PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07H 21/04, C07K 14/00, 16/00, C12N 1/20, 15/00, 15/09, 15/63

A1

(11) International Publication Number: WO 98/06737

(43) International Publication Date: 19 February 1998 (19.02.98)

(21) International Application Number: PCT/US97/14593

(22) International Filing Date: 15 August 1997 (15.08.97)

(30) Priority Data:

60/024,050	16 August 1996 (16.08.96)	US
08/706,408	30 August 1996 (30.08.96)	US

(60) Parent Applications or Grants
(63) Related by Continuation

US	60/024,050 (CIP)
Filed on	16 August 1996 (16.08.96)
US	08/706,408 (CIP)
Filed on	30 August 1996 (30.08.96)

(71) Applicants *(for all designated States except US)*: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612-3550 (US). STATE OF OREGON, acting by and through THE STATE BOARD OF HIGHER EDUCATION on behalf of THE UNIVERSITY OF OREGON [US/US]; Eugene, OR 97403 (US). AURORA BIOSCIENCES [US/US]; 11149 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: TSIEN, Roger, Y. [US/US]; 8535 Nottingham Place, La Jolla, CA 92037 (US). CUBITT, Andrew, B. [GB/GB]; 11083 Caminito Encanto, San Diego, CA 92131 (US). HEIM, Roger [CH/CH]; 530 Stratford Court #309B, Del Mar, CA 92014 (US). ORMO, Mats, F. [SE/SE]; Juristvägen 18, S-141 73 Huddinge (SE). REMINGTON, James, S. [US/US]; 2100 River Loop 1, Eugene, OR 97404 (US).

(74) Agent: HAILE, Lisa, A.; Fish & Richardson P.C., Suite 1400, 4225 Executive Square, La Jolla, CA 92037 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS

(57) Abstract

Engineered fluorescent proteins, nucleic acids encoding them and methods of use.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS

BACKGROUND OF THE INVENTION

This application claims the benefit of the earlier filing date of a United States provisional patent application serial number 60/024,050 filed on August 16, 1996 entitled "Long Wavelength Mutant Fluorescent Proteins" and patent application serial number 08/706,408filed on August 30, 1996 entitled "Long Wavelength Engineered Fluorescent Proteins," both of which are herein incorporated by reference.

This invention was made in part with Government support under grant no. MCB 9418479 awarded by the National Science Foundation. The Government may have rights in this invention.

Fluorescent molecules are attractive as reporter molecules in many assay systems because of their high sensitivity and ease of quantification. Recently, fluorescent proteins have been the focus of much attention because they can be produced *in vivo* by biological systems, and can be used to trace intracellular events without the need to be introduced into the cell through microinjection or permeabilization. The green fluorescent protein of *Aequorea victoria* is particularly interesting as a fluorescent protein. A cDNA for the protein has been cloned. (D.C. Prasher et al., "Primary structure of the *Aequorea victoria* green-fluorescent protein," *Gene* (1992) 111:229-33.) Not only can the primary amino acid sequence of the protein be expressed from the cDNA, but the expressed protein can fluoresce. This indicates that the protein can undergo the cyclization and oxidation believed to be necessary for fluorescence. *Aequorea* green fluorescent protein ("GFP") is a stable, proteolysis-resistant single chain of 238 residues and has two absorption maxima at around 395 and 475 nm. The relative amplitudes of these two peaks is sensitive to environmental factors (W. W. Ward. *Bioluminescence and Chemiluminescence* (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman *Biochemistry* 21:4535-4540 (1982); W. W. Ward et al. *Photochem. Photobiol.* 35:803-808 (1982)) and illumination history (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995)), presumably reflecting two or more ground states. Excitation at the primary absorption peak of 395 nm yields an emission maximum at 508 nm with a quantum yield of 0.72-0.85 (O. Shimomura and F.H. Johnson *J. Cell. Comp. Physiol.* 59:223 (1962);

J. G. Morin and J. W. Hastings, *J. Cell. Physiol.* 77:313 (1971); H. Morise et al. *Biochemistry* 13:2656 (1974); W. W. Ward *Photochem. Photobiol. Reviews* (Smith, K. C. ed.) 4:1 (1979); A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995); D. C. Prasher *Trends Genet.* 11:320-323 (1995); M. Chalfie *Photochem. Photobiol.* 62:651-656 (1995); W. W. Ward. *Bioluminescence and Chemiluminescence* (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman *Biochemistry* 21:4535-4540 (1982); W. W. Ward et al. *Photochem. Photobiol.* 35:803-808 (1982)). The fluorophore results from the autocatalytic cyclization of the polypeptide backbone between residues Ser^{65} and Gly^{67} and oxidation of the □-ß bond of Tyr^{66} (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995); C. W. Cody et al. *Biochemistry* 32:1212-1218 (1993); R. Heim et al. *Proc. Natl. Acad. Sci.* USA 91:12501-12504 (1994)). Mutation of Ser^{65} to Thr (S65T) simplifies the excitation spectrum to a single peak at 488 nm of enhanced amplitude (R. Heim et al. *Nature* 373:664-665 (1995)), which no longer gives signs of conformational isomers (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995)).

Fluorescent proteins have been used as markers of gene expression, tracers of cell lineage and as fusion tags to monitor protein localization within living cells. (M. Chalfie et al., "Green fluorescent protein as a marker for gene expression," *Science* 263:802-805; A.B. Cubitt et al., "Understanding, improving and using green fluorescent proteins," *TIBS 20*, November 1995, pp. 448-455. U.S. patent 5,491,084, M. Chalfie and D. Prasher. Furthermore, engineered versions of *Aequorea* green fluorescent protein have been identified that exhibit altered fluorescence characteristics, including altered excitation and emission maxima, as well as excitation and emission spectra of different shapes. (R. Heim et al., "Wavelength mutations and posttranslational autoxidation of green fluorescent protein," *Proc. Natl. Acad. Sci. USA*, (1994) 91:12501-04; R. Heim et al., "Improved green fluorescence," *Nature* (1995) 373:663-665.) These properties add variety and utility to the arsenal of biologically based fluorescent indicators.

There is a need for engineered fluorescent proteins with varied fluorescent properties.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1B. (A) Schematic drawing of the backbone of GFP produced by

Molscript (J.P. Kraulis, *J. Appl. Cryst.*, 24:946 (1991)). The chromophore is shown as a ball and stick model. (B) Schematic drawing of the overall fold of GFP. Approximate residue numbers mark the beginning and ending of the secondary structure elements.

Figs. 2A-2C. (A) Stereo drawing of the chromophore and residues in the immediate vicinity. Carbon atoms are drawn as open circles, oxygen is filled and nitrogen is shaded. Solvent molecules are shown as isolated filled circles. (B) Portion of the final $2F_o$-F_c electron density map contoured at 1.0 □, showing the electron density surrounding the chromophore. (C) Schematic diagram showing the first and second spheres of coordination of the chromophore. Hydrogen bonds are shown as dashed lines and have the indicated lengths in Å. Inset: proposed structure of the carbinolamine intermediate that is presumably formed during generation of the chromophore.

Fig. 3 depicts the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of an *Aequorea* green fluorescent protein.

Fig. 4 depicts the nucleotide sequence (SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) of the engineered *Aequorea*-related fluorescent protein S65G/S72A/T203Y utilizing preferred mammalian codons and optimal Kozak sequence.

Figs. 5-1 to 5-28 present the coordinates for the crystal structure of *Aequorea*-related green fluorescent protein S65T.

Fig. 6 shows the fluorescence excitation and emission spectra for engineered fluorescent proteins 20A and 10C (Table F). The vertical line at 528 nm compares the emission maxima of 10C, to the left of the line, and 20A, to the right of the line.

SUMMARY OF THE INVENTION

This invention provides functional engineered fluorescent proteins with varied fluorescence characteristics that can be easily distinguished from currently existing green and blue fluorescent proteins. Such engineered fluorescent proteins enable the simultaneous measurement of two or more processes within cells and can be used as fluorescence energy donors or acceptors when used to monitor protein-protein interactions through FRET. Longer wavelength engineered fluorescent proteins are particularly useful because photodynamic toxicity and auto-fluorescence of cells are significantly reduced at longer wavelengths. In particular, the introduction of the substitution T203X, wherein X is an aromatic amino acid, results in an increase in the excitation and emission wavelength

maxima of *Aequorea*-related fluorescent proteins.

In one aspect, this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution located no more than about 0.5 nm from the chromophore of the engineered fluorescent protein, wherein the substitution alters the electronic environment of the chromophore, whereby the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

In one aspect this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at T203 and, in particular, T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein. In one embodiment, the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I. In another embodiment, the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S65G/V68L/Q69K/S72A/T203Y; S72A/S65G/V68L/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W. In another embodiment, the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W. In another embodiment, the amino acid sequence further comprises a mutation from Table A. In another embodiment, the amino acid sequence further comprises a folding mutation. In another embodiment, the nucleotide sequence encoding the protein differs from the nucleotide sequence of SEQ ID NO:1 by the substitution of at least one codon by a preferred mammalian codon. In another embodiment, the nucleic acid molecule encodes a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

In another aspect, this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green

fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein. In one embodiment, amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,
V61X, wherein X is selected from F, Y, H and C,
T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,
V68X, wherein X is selected from F, Y and H,
Q69X, wherein X is selected from K, R, E and G,
Q94X, wherein X is selected from D, E, H, K and N,
N121X, wherein X is selected from F, H, W and Y,
Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,
H148X, wherein X is selected from F, Y, N, K, Q and R,
V150X, wherein X is selected from F, Y and H,
F165X, wherein X is selected from H, Q, W and Y,
I167X, wherein X is selected from F, Y and H,
Q183X, wherein X is selected from H, Y, E and K,
N185X, wherein X is selected from D, E, H, K and Q,
L220X, wherein X is selected from H, N, Q and T,
E222X, wherein X is selected from N and Q, or
V224X, wherein X is selected from H, N, Q, T, F, W and Y.

In a further aspect, this invention provides an expression vector comprising expression control sequences operatively linked to any of the aforementioned nucleic acid molecules. In a further aspect, this invention provides a recombinant host cell comprising the aforementioned expression vector.

In another aspect, this invention provides a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution located no more than about 0.5 nm from the chromophore of the engineered fluorescent protein, wherein the substitution alters the

electronic environment of the chromophore, whereby the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

In another aspect, this invention provides a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution at T203, and in particular, T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein. In one embodiment, the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I. In another embodiment, the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W. In another embodiment, the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W. In another embodiment, the amino acid sequence further comprises a folding mutation. In another embodiment, the engineered fluorescent protein is part of a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

In another aspect this invention provides a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222, or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

In another aspect, this invention provides a fluorescently labelled antibody comprising an antibody coupled to any of the aforementioned functional engineered fluorescent proteins. In one embodiment, the fluorescently labelled antibody is a fusion protein wherein the fusion protein comprises the antibody fused to the functional engineered fluorescent protein.

In another aspect, this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding an antibody fused to a nucleotide sequence encoding a

functional engineered fluorescent protein of this invention.

In another aspect, this invention provides a fluorescently labelled nucleic acid probe comprising a nucleic acid probe coupled to a functional engineered fluorescent protein whose amino acid sequence of this invention. The fusion can be through a linker peptide.

In another aspect, this invention provides a method for determining whether a mixture contains a target comprising contacting the mixture with a fluorescently labelled probe comprising a probe and a functional engineered fluorescent protein of this invention; and determining whether the target has bound to the probe. In one embodiment, the target molecule is captured on a solid matrix.

In another aspect, this invention provides a method for engineering a functional engineered fluorescent protein having a fluorescent property different than *Aequorea* green fluorescent protein, comprising substituting an amino acid that is located no more than 0.5 nm from any atom in the chromophore of an *Aequorea*-related green fluorescent protein with another amino acid; whereby the substitution alters a fluorescent property of the protein. In one embodiment, the amino acid substitution alters the electronic environment of the chromophore.

In another aspect, this invention provides a method for engineering a functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein comprising substituting amino acids in a loop domain of an *Aequorea*-related green fluorescent protein with amino acids so as to create a consensus sequence for phosphorylation or for proteolysis.

In another aspect, this invention provides a method for producing fluorescence resonance energy transfer comprising providing a donor molecule comprising a functional engineered fluorescent protein this invention; providing an appropriate acceptor molecule for the fluorescent protein; and bringing the donor molecule and the acceptor molecule into sufficiently close contact to allow fluorescence resonance energy transfer.

In another aspect, this invention provides a method for producing fluorescence resonance energy transfer comprising providing an acceptor molecule comprising a functional engineered fluorescent protein of this invention; providing an appropriate donor molecule for the fluorescent protein; and bringing the donor molecule and the acceptor molecule into sufficiently close contact to allow fluorescence resonance energy

transfer. In one embodiment, the donor molecule is a engineered fluorescent protein whose amino acid sequence comprises the substitution T203I and the acceptor molecule is an engineered fluorescent protein whose amino acid sequence comprises the substitution T203X. wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

In another aspect, this invention provides a crystal of a protein comprising a fluorescent protein with an amino acid sequence substantially identical to SEQ ID NO: 2, wherein said crystal diffracts with at least a 2.0 to 3.0 angstrom resolution.

In another embodiment, this invention provides computational method of designing a fluoresent protein comprising determining from a three dimensional model of a crystallized fluorescent protein comprising a fluorescent protein with a bound ligand, at least one interacting amino acid of the fluorescent protein that interacts with at least one first chemical moiety of the ligand, and selecting at least one chemical modification of the first chemical moiety to produce a second chemical moiety with a structure to either decrease or increase an interaction between the interacting amino acid and the second chemical moiety compared to the interaction between the interacting amino acid and the first chemical moiety.

In another embodiment, this invention provides a computational method of modeling the three dimensional structure of a fluorescent protein comprising determining a three dimensional relationship between at least two atoms listed in the atomic coordinates of Figs. 5-1 to 5-28.

In another embodiment, this invention provides a device comprising a storage device and, stored in the device, at least 10 atomic coordinates selected from the atomic coordinates listed in Figs. 5-1 to 5-28. In one embodiment, the storage device is a computer readable device that stores code that receives as input the atomic coordinates. In another embodiment, the computer readable device is a floppy disk or a hard drive.

DETAILED DESCRIPTION OF THE INVENTION

I. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which

this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. For purposes of the present invention, the following terms are defined below.

"Binding pair" refers to two moieties (e.g. chemical or biochemical) that have an affinity for one another. Examples of binding pairs include antigen/antibodies, lectin/avidin, target polynucleotide/probe oligonucleotide, antibody/anti-antibody, receptor/ligand, enzyme/ligand and the like. "One member of a binding pair" refers to one moiety of the pair, such as an antigen or ligand.

"Nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and, unless otherwise limited, encompasses known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides. It will be understood that when a nucleic acid molecule is represented by a DNA sequence, this also includes RNA molecules having the corresponding RNA sequence in which "U" replaces "T."

"Recombinant nucleic acid molecule" refers to a nucleic acid molecule which is not naturally occurring, and which comprises two nucleotide sequences which are not naturally joined together. Recombinant nucleic acid molecules are produced by artificial recombination, e.g., genetic engineering techniques or chemical synthesis.

Reference to a nucleotide sequence "encoding" a polypeptide means that the sequence, upon transcription and translation of mRNA, produces the polypeptide. This includes both the coding strand, whose nucleotide sequence is identical to mRNA and whose sequence is usually provided in the sequence listing, as well as its complementary strand, which is used as the template for transcription. As any person skilled in the art recognizes, this also includes all degenerate nucleotide sequences encoding the same amino acid sequence. Nucleotide sequences encoding a polypeptide include sequences containing introns.

"Expression control sequences" refers to nucleotide sequences that regulate the expression of a nucleotide sequence to which they are operatively linked. Expression control sequences are "operatively linked" to a nucleotide sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleotide sequence. Thus, expression control sequences can include appropriate

promoters, enhancers, transcription terminators, a start codon (i.e., ATG) in front of a protein-encoding gene, splicing signals for introns, maintenance of the correct reading frame of that gene to permit proper translation of the mRNA, and stop codons.

"Naturally-occurring" as used herein, as applied to an object, refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences, such as when the appropriate molecules (e.g., inducers and polymerases) are bound to the control or regulatory sequence(s).

"Control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding and non-coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

"Isolated polynucleotide" refers a polynucleotide of genomic, cDNA, or synthetic origin or some combination there of, which by virtue of its origin the "isolated polynucleotide" (1) is not associated with the cell in which the "isolated polynucleotide" is found in nature, or (2) is operably linked to a polynucleotide which it is not linked to in nature.

"Polynucleotide" refers to a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

The term "probe" refers to a substance that specifically binds to another substance (a "target"). Probes include, for example, antibodies, nucleic acids, receptors and

their ligands.

"Modulation" refers to the capacity to either enhance or inhibit a functional property of biological activity or process (e.g., enzyme activity or receptor binding); such enhancement or inhibition may be contingent on the occurrence of a specific event, such as activation of a signal transduction pathway, and/or may be manifest only in particular cell types.

The term "modulator" refers to a chemical (naturally occurring or non-naturally occurring), such as a synthetic molecule (e.g., nucleic acid, protein, non-peptide, or organic molecule), or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues. Modulators can be evaluated for potential activity as inhibitors or activators (directly or indirectly) of a biological process or processes (e.g., agonist, partial antagonist, partial agonist, inverse agonist, antagonist, antineoplastic agents, cytotoxic agents, inhibitors of neoplastic transformation or cell proliferation, cell proliferation-promoting agents, and the like) by inclusion in screening assays described herein. The activity of a modulator may be known, unknown or partially known.

The term "test chemical" refers to a chemical to be tested by one or more screening method(s)of the invention as a putative modulator. A test chemical is usually not known to bind to the target of interest. The term "control test chemical" refers to a chemical known to bind to the target (e.g., a known agonist, antagonist, partial agonist or inverse agonist). Usually, various predetermined concentrations of test chemicals are used for screening, such as .01 μM, .1 μM, 1.0 μM, and 10.0 μM.

The term "target" refers to a biochemical entity involved a biological process. Targets are typically proteins that play a useful role in the physiology or biology of an organism. A therapeutic chemical binds to target to alter or modulate its function. As used herein targets can include cell surface receptors, G-proteins, kinases, ion channels, phopholipases and other proteins mentioned herein.

The term "label" refers to a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P, fluorescent dyes, fluorescent proteins, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, dioxigenin, or haptens and proteins for which antisera or monoclonal antibodies are available. For example, polypeptides of this invention can be made as detectible labels, by e.g., incorporating a them as into a polypeptide, and

used to label antibodies specifically reactive with the polypeptide. A label often generates a measurable signal, such as radioactivity, fluorescent light or enzyme activity, which can be used to quantitate the amount of bound label.

The term "nucleic acid probe" refers to a nucleic acid molecule that binds to a specific sequence or sub-sequence of another nucleic acid molecule. A probe is preferably a nucleic acid molecule that binds through complementary base pairing to the full sequence or to a sub-sequence of a target nucleic acid. It will be understood that probes may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. Probes are preferably directly labelled as with isotopes, chromophores, lumiphores, chromogens, fluorescent proteins, or indirectly labelled such as with biotin to which a streptavidin complex may later bind. By assaying for the presence or absence of the probe, one can detect the presence or absence of the select sequence or sub-sequence.

A "labeled nucleic acid probe" is a nucleic acid probe that is bound, either covalently, through a linker, or through ionic, van der Waals or hydrogen bonds to a label such that the presence of the probe may be detected by detecting the presence of the label bound to the probe.

The terms "polypeptide" and "protein" refers to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The term "recombinant protein" refers to a protein that is produced by expression of a nucleotide sequence encoding the amino acid sequence of the protein from a recombinant DNA molecule.

The term "recombinant host cell" refers to a cell that comprises a recombinant nucleic acid molecule. Thus, for example, recombinant host cells can express genes that are not found within the native (non-recombinant) form of the cell.

The terms "isolated" "purified" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein or nucleic acid molecule which is the predominant protein or nucleic acid species present in a preparation is substantially purified. Generally, an isolated

protein or nucleic acid molecule will comprise more than 80% of all macromolecular species present in the preparation. Preferably, the protein is purified to represent greater than 90% of all macromolecular species present. More preferably the protein is purified to greater than 95%, and most preferably the protein is purified to essential homogeneity, wherein other macromolecular species are not detected by conventional techniques.

The term "naturally-occurring" as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "antibody" refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, which specifically bind and recognize an analyte (antigen). The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Antibodies exist, e.g., as intact immunoglobulins or as a number of well characterized fragments produced by digestion with various peptidases. This includes, e.g., Fab' and $F(ab)'_2$ fragments. The term "antibody," as used herein, also includes antibody fragments either produced by the modification of whole antibodies or those synthesized *de novo* using recombinant DNA methodologies.

The term "immunoassay" refers to an assay that utilizes an antibody to specifically bind an analyte. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the analyte.

The term "identical" in the context of two nucleic acid or polypeptide sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. When percentage of sequence identity is used in reference to proteins or peptides it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acids residues are substituted for other amino acid residues with similar chemical properties (e.g. charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for

making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, *e.g.*, according to known algorithm. *See, e.g.*, Meyers and Miller, Computer Applic. Biol. Sci., 4: 11-17 (1988); Smith and Waterman (1981) *Adv. Appl. Math.* 2: 482; Needleman and Wunsch (1970) *J. Mol. Biol.* 48: 443; Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444; Higgins and Sharp (1988) *Gene*, 73: 237-244 and Higgins and Sharp (1989) *CABIOS* 5: 151-153; Corpet, *et al.* (1988) *Nucleic Acids Research* 16, 10881-90; Huang, *et al.* (1992) *Computer Applications in the Biosciences* 8, 155-65, and Pearson, *et al.* (1994) *Methods in Molecular Biology* 24, 307-31. Alignment is also often performed by inspection and manual alignment.

"Conservatively modified variations" of a particular nucleic acid sequence refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance, the codons CGU, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of "conservatively modified variations." Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques. Accordingly, each "silent variation" of a nucleic acid which encodes a polypeptide is implicit in each described sequence. Furthermore, one of skill will recognize that individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are "conservatively modified variations" where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative amino acid substitutions

providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);

2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

The term "complementary" means that one nucleic acid molecule has the sequence of the binding partner of another nucleic acid molecule. Thus, the sequence 5'-ATGC-3' is complementary to the sequence 5'-GCAT-3'.

An amino acid sequence or a nucleotide sequence is "substantially identical" or "substantially similar" to a reference sequence if the amino acid sequence or nucleotide sequence has at least 80% sequence identity with the reference sequence over a given comparison window. Thus, substantially similar sequences include those having, for example, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity or at least 99% sequence identity. Two sequences that are identical to each other are, of course, also substantially identical.

A subject nucleotide sequence is "substantially complementary" to a reference nucleotide sequence if the complement of the subject nucleotide sequence is substantially identical to the reference nucleotide sequence.

The term "stringent conditions" refers to a temperature and ionic conditions used in nucleic acid hybridization. Stringent conditions are sequence dependent and are different under different environmental parameters. Generally, stringent conditions are selected to be about 5□C to 20□C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe.

The term "allelic variants" refers to polymorphic forms of a gene at a particular genetic locus, as well as cDNAs derived from mRNA transcripts of the genes and the polypeptides encoded by them.

The term "preferred mammalian codon" refers to the subset of codons from

among the set of codons encoding an amino acid that are most frequently used in proteins expressed in mammalian cells as chosen from the following list:

Amino Acid	Preferred codons for high level mammalian expression
Gly	GGC,GGG
Glu	GAG
Asp	GAC
Val	GUG,GUC
Ala	GCC,GCU
Ser	AGC,UCC
Lys	AAG
Asn	AAC
Met	AUG
Ile	AUC
Thr	ACC
Trp	UGG
Cys	UGC
Tyr	UAU,UAC
Leu	CUG
Phe	UUC
Arg	CGC,AGG,AGA
Gln	CAG
His	CAC
Pro	CCC

Fluorescent molecules are useful in fluorescence resonance energy transfer ("FRET"). FRET involves a donor molecule and an acceptor molecule. To optimize the efficiency and detectability of FRET between a donor and acceptor molecule, several factors need to be balanced. The emission spectrum of the donor should overlap as much as possible with the excitation spectrum of the acceptor to maximize the overlap integral. Also, the quantum yield of the donor moiety and the extinction coefficient of the acceptor should likewise be as high as possible to maximize R_0, the distance at which energy transfer efficiency is 50%. However, the excitation spectra of the donor and acceptor should overlap as little as possible so that a wavelength region can be found at which the donor can be excited efficiently without directly exciting the acceptor. Fluorescence arising from direct excitation of the acceptor is difficult to distinguish from fluorescence arising from FRET. Similarly, the emission spectra of the donor and acceptor should overlap as little as possible so that the two emissions can be clearly distinguished. High fluorescence quantum yield of

the acceptor moiety is desirable if the emission from the acceptor is to be measured either as the sole readout or as part of an emission ratio. One factor to be considered in choosing the donor and acceptor pair is the efficiency of fluorescence resonance energy transfer between them. Preferably, the efficiency of FRET between the donor and acceptor is at least 10%, more preferably at least 50% and even more preferably at least 80%.

The term "fluorescent property" refers to the molar extinction coefficient at an appropriate excitation wavelength, the fluorescence quantum efficiency, the shape of the excitation spectrum or emission spectrum, the excitation wavelength maximum and emission wavelength maximum, the ratio of excitation amplitudes at two different wavelengths, the ratio of emission amplitudes at two different wavelengths, the excited state lifetime, or the fluorescence anisotropy. A measurable difference in any one of these properties between wild-type *Aequorea* GFP and the mutant form is useful. A measurable difference can be determined by determining the amount of any quantitative fluorescent property, e.g., the amount of fluorescence at a particular wavelength, or the integral of fluorescence over the emission spectrum. Determining ratios of excitation amplitude or emission amplitude at two different wavelengths ("excitation amplitude ratioing" and "emission amplitude ratioing", respectively) are particularly advantageous because the ratioing process provides an internal reference and cancels out variations in the absolute brightness of the excitation source, the sensitivity of the detector, and light scattering or quenching by the sample.

II. LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS

A. Fluorescent Proteins

As used herein, the term "fluorescent protein" refers to any protein capable of fluorescence when excited with appropriate electromagnetic radiation. This includes fluorescent proteins whose amino acid sequences are either naturally occurring or engineered (i.e., analogs or mutants). Many cnidarians use green fluorescent proteins ("GFPs") as energy-transfer acceptors in bioluminescence. A "green fluorescent protein," as used herein, is a protein that fluoresces green light. Similarly, "blue fluorescent proteins" fluoresce blue light and "red fluorescent proteins" fluoresce red light. GFPs have been isolated from the Pacific Northwest jellyfish, *Aequorea victoria*, the sea pansy, *Renilla reniformis*, and *Phialidium gregarium*. W.W. Ward et al., *Photochem. Photobiol.*, 35:803-808 (1982); L.D. Levine et al., *Comp. Biochem. Physiol.*, 72B:77-85 (1982).

A variety of *Aequorea*-related fluorescent proteins having useful excitation and emission spectra have been engineered by modifying the amino acid sequence of a naturally occurring GFP from *Aequorea victoria*. (D.C. Prasher et al., *Gene*, 111:229-233 (1992); R. Heim et al., *Proc. Natl. Acad. Sci., USA*, 91:12501-04 (1994); U.S. patent application 08/337,915, filed November 10, 1994; International application PCT/US95/14692, filed 11/10/95.)

As used herein, a fluorescent protein is an "*Aequorea*-related fluorescent protein" if any contiguous sequence of 150 amino acids of the fluorescent protein has at least 85% sequence identity with an amino acid sequence, either contiguous or non-contiguous, from the 238 amino-acid wild-type *Aequorea* green fluorescent protein of Fig. 3 (SEQ ID NO:2). More preferably, a fluorescent protein is an *Aequorea*-related fluorescent protein if any contiguous sequence of 200 amino acids of the fluorescent protein has at least 95% sequence identity with an amino acid sequence, either contiguous or non-contiguous, from the wild type *Aequorea* green fluorescent protein of Fig. 3 (SEQ ID NO:2). Similarly, the fluorescent protein may be related to *Renilla* or *Phialidium* wild-type fluorescent proteins using the same standards.

Aequorea-related fluorescent proteins include, for example and without limitation, wild-type (native) *Aequorea victoria* GFP (D.C. Prasher et al., "Primary structure of the *Aequorea victoria* green fluorescent protein," *Gene*, (1992) 111:229-33), whose nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) are presented in Fig. 3; allelic variants of this sequence, e.g., Q80R, which has the glutamine

residue at position 80 substituted with arginine (M. Chalfie et al., *Science*, (1994) 263:802-805); those engineered *Aequorea*-related fluorescent proteins described herein, e.g., in Table A or Table F, variants that include one or more folding mutations and fragments of these proteins that are fluorescent, such as *Aequorea* green fluorescent protein from which the two amino-terminal amino acids have been removed. Several of these contain different aromatic amino acids within the central chromophore and fluoresce at a distinctly shorter wavelength than wild type species. For example, engineered proteins P4 and P4-3 contain (in addition to other mutations) the substitution Y66H, whereas W2 and W7 contain (in addition to other mutations) Y66W. Other mutations both close to the chromophore region of the protein and remote from it in primary sequence may affect the spectral properties of GFP and are listed in the first part of the table below.

TABLE A

Clone	Mutation(s)	Excitation max (nm)	Emission max (nm)	Extinct. Coeff. ($M^{-1}cm^{-1}$)	Quantum yield
Wild type	None	395 (475)	508	21,000 (7,150)	0.77
P4	Y66H	383	447	13,500	0.21
P4-3	Y66H Y145F	381	445	14,000	0.38
W7	Y66W N146I M153T V163A N212K	433 (453)	475 (501)	18,000 (17,100)	0.67
W2	Y66W I123V Y145H H148R M153T V163A N212K	432 (453)	480	10,000 (9,600)	0.72
S65T	S65T	489	511	39,200	0.68
P4-1	S65T M153A	504 (396)	514	14,500 (8,600)	0.53

	K238E		
S65A	S65A	471	504
S65C	S65C	479	507
S65L	S65L	484	510
Y66F	Y66F	360	442
Y66W	Y66W	458	480

Additional mutations in *Aequorea*-related fluorescent proteins, referred to as "folding mutations," improve the ability of fluorescent proteins to fold at higher temperatures, and to be more fluorescent when expressed in mammalian cells, but have little or no effect on the peak wavelengths of excitation and emission. It should be noted that these may be combined with mutations that influence the spectral properties of GFP to produce proteins with altered spectral and folding properties. Folding mutations include: F64L, V68L, S72A, and also T44A, F99S, Y145F, N146I, M153T or A, V163A, I167T, S175G, S205T and N212K.

As used herein, the term "loop domain" refers to an amino acid sequence of an *Aequorea*-related fluorescent protein that connects the amino acids involved in the secondary structure of the eleven strands of the □-barrel or the central □-helix (residues 56-72) (see Fig. 1A and 1B).

As used herein, the "fluorescent protein moiety" of a fluorescent protein is that portion of the amino acid sequence of a fluorescent protein which, when the amino acid sequence of the fluorescent protein substrate is optimally aligned with the amino acid sequence of a naturally occurring fluorescent protein, lies between the amino terminal and carboxy terminal amino acids, inclusive, of the amino acid sequence of the naturally occurring fluorescent protein.

It has been found that fluorescent proteins can be genetically fused to other target proteins and used as markers to identify the location and amount of the target protein produced. Accordingly, this invention provides fusion proteins comprising a fluorescent protein moiety and additional amino acid sequences. Such sequences can be, for example, up to about 15, up to about 50, up to about 150 or up to about 1000 amino acids long. The

fusion proteins possess the ability to fluoresce when excited by electromagnetic radiation. In one embodiment, the fusion protein comprises a polyhistidine tag to aid in purification of the protein.

B. Use Of The Crystal Structure Of Green Fluorescent Protein To Design Mutants Having Altered Fluorescent Characteristics

Using X-ray crystallography and computer processing, we have created a model of the crystal structure of *Aequorea* green fluorescent protein showing the relative location of the atoms in the molecule. This information is useful in identifying amino acids whose substitution alters fluorescent properties of the protein.

Fluorescent characteristics of *Aequorea*-related fluorescent proteins depend, in part, on the electronic environment of the chromophore. In general, amino acids that are within about 0.5 nm of the chromophore influence the electronic environment of the chromophore. Therefore, substitution of such amino acids can produce fluorescent proteins with altered fluorescent characteristics. In the excited state, electron density tends to shift from the phenolate towards the carbonyl end of the chromophore. Therefore, placement of increasing positive charge near the carbonyl end of the chromophore tends to decrease the energy of the excited state and cause a red-shift in the absorbance and emission wavelength maximum of the protein. Decreasing positive charge near the carbonyl end of the chromophore tends to have the opposte effect, causing a blue-shift in the protein's wavelengths.

Amino acids with charged (ionized D, E, K, and R), dipolar (H, N, Q, S, T, and uncharged D, E and K), and polarizable side groups (e.g., C, F, H, M, W and Y) are useful for altering the electronic environment of the chromophore, especially when they substitute an amino acid with an uncharged, nonpolar or non-polarizable side chain. In general, amino acids with polarizable side groups alter the electronic environment least, and, consequently, are expected to cause a comparatively smaller change in a fluorescent property. Amino acids with charged side groups alter the environment most, and, consequently, are expected to cause a comparatively larger change in a fluorescent property. However, amino acids with charged side groups are more likely to disrupt the structure of the protein and to prevent proper folding if buried next to the chromophore without any

additional solvation or salt bridging. Therefore charged amino acids are most likely to be tolerated and to give useful effects when they replace other charged or highly polar amino acids that are already solvated or involved in salt bridges. In certain cases, where substitution with a polarizable amino acid is chosen, the structure of the protein may make selection of a larger amino acid, e.g., W, less appropriate. Alternatively, positions occupied by amino acids with charged or polar side groups that are unfavorably oriented may be substituted with amino acids that have less charged or polar side groups. In another alternative, an amino acid whose side group has a dipole oriented in one direction in the protein can be substituted with an amino acid having a dipole oriented in a different direction.

More particularly, Table B lists several amino acids located within about 0.5 nm from the chromophore whose substitution can result in altered fluorescent characteristics. The table indicates, underlined, preferred amino acid substitutions at the indicated location to alter a fluorescent characteristic of the protein. In order to introduce such substitutions, the table also provides codons for primers used in site- directed mutagenesis involving amplification. These primers have been selected to encode economically the preferred amino acids, but they encode other amino acids as well, as indicated, or even a stop codon, denoted by Z. In introducing substitutions using such degenerate primers the most efficient strategy is to screen the collection to identify mutants with the desired properties and then sequence their DNA to find out which of the possible substitutions is responsible. Codons are shown in double-stranded form with sense strand above, antisense strand below. In nucleic acid sequences, R=(A or g); Y=(C or T); M=(A or C); K=(g or T); S=(g or C); W=(A or T); H=(A, T, or C); B=(g, T, or C); V=(g, A, or C); D=(g, A, or T); N=(A, C, g, or T).

TABLE B

Original position and presumed role		Change to	Codon
L42	Aliphatic residue near C=N of chromophore	CFHLQRWYZ	5'YDS 3' 3'RHS 5'
V61	Aliphatic residue near central -CH= of chromophore	FYHCLR	YDC RHg

	T62	Almost directly above center of chromophore bridge	AVFS	KYC MRg
			DEHKNQ	VAS BTS
			FYHCLR	YDC RHg
	V68	Aliphatic residue near carbonyl and G67	FYHL	YWC RWg
	N121	Near C-N site of ring closure between T65 and G67	CFHLQRWYZ	YDS RHS
	Y145	Packs near tyrosine ring of chromophore	WCFL	TKS AMS
			DEHNKQ	VAS BTS
	H148	H-bonds to phenolate oxygen	FYNI	WWC WWg
			KQR	MRg KYC
	V150	Aliphatic residue near tyrosine ring of chromophore	FYHL	YWC RWg
	F165	Packs near tyrosine ring	CHQRWYZ	YRS RYS
	I167	Aliphatic residue near phenolate; I167T has effects	FYHL	YWC RWg
	T203	H-bonds to phenolic oxygen of chromophore	FHLQRWYZ	YDS RHS
	E222	Protonation regulates ionization of chromophore	HKNQ	MAS KTS

Examples of amino acids with polar side groups that can be substituted with polarizable side groups include, for example, those in Table C.

TABLE C

Original position and presumed role		Change to	Codon
Q69	Terminates chain of H-bonding waters	KREG	RRg YYC
Q94	H-bonds to carbonyl terminus of chromophore	DEHKNQ	VAS BTS
Q183	Bridges Arg96 and center of chromophore bridge	HY	YAC RTG
		EK	RAg YTC
N185	Part of H-bond network near carbonyl of chromophore	DEHNKQ	VAS BTS

In another embodiment, an amino acid that is close to a second amino acid within about 0.5 nm of the chromophore can, upon substitution, alter the electronic properties of the second amino acid, in turn altering the electronic environment of the chromphore. Table D presents two such amino acids. The amino acids, L220 and V224, are close to E222 and oriented in the same direction in the □ pleated sheet.

TABLE D

Original position and presumed role		Change to	Codon
L220	Packs next to Glu222; to make GFP pH sensitive	HKNPQT	MMS KKS
V224	Packs next to Glu222; to make GFP pH sensitive	HKNPQT	MMS KKS
		CFHLQRWYZ	YDS RHS

One embodiment of the invention includes a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at Q69, wherein the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein. Preferably, the substitution at Q69 is selected from the group of K, R, E and G. The Q69 substitution can be combined with other mutations to improve the properties of the protein, such as a functional mutation at S65.

One embodiment of the invention includes a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at E222, but not including E222G, wherein the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein. Preferably, the substitution at E222 is selected from the group of N and Q. The E222 substitution can be combined with other mutations to improve the properties of the protein, such as a functional mutation at F64.

One embodiment of the invention includes a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at Y145, wherein the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

Preferably, the substitution at Y145 is selected from the group of W, C, F, L, E, H, K and Q. The Y145 substitution can be combined with other mutations to improve the properties of the protein, such as a Y66.

The invention also includes computer related embodiments, including computational methods of using the crystal coordinates for designing new fluorescent protein mutations and devices for storing the crystal data, including coordinates. For instance the invention includes a device comprising a storage device and, stored in the device, at least 10 atomic coordinates selected from the atomic coordinates listed in Figs. 5-1 to 5-28. More coordinates can be storage depending of the complexity of the calculations or the objective of using the coordinates (e.g. about 100, 1,000, or more coordinates). For example, larger numbers of coordinates will be desirable for more detailed representations of fluorescent protein structure. Typically, the storage device is a computer readable device that stores code that it receives as input the atomic coordinates. Although, other storage meand as known in the art are contemplated. The computer readable device can be a floppy disk or a hard drive.

C. Production Of Long Wavelength Engineered Fluorescent Proteins

Recombinant production of a fluorescent protein involves expressing a nucleic acid molecule having sequences that encode the protein.

In one embodiment, the nucleic acid encodes a fusion protein in which a single polypeptide includes the fluorescent protein moiety within a longer polypeptide. The longer polypeptide can include a second functional protein, such as FRET partner or a protein having a second function (e.g., an enzyme, antibody or other binding protein). Nucleic acids that encode fluorescent proteins are useful as starting materials.

The fluorescent proteins can be produced as fusion proteins by recombinant DNA technology. Recombinant production of fluorescent proteins involves expressing nucleic acids having sequences that encode the proteins. Nucleic acids encoding fluorescent proteins can be obtained by methods known in the art. Fluorescent proteins can be made by site-specific mutagenesis of other nucleic acids encoding fluorescent proteins, or by random mutagenesis caused by increasing the error rate of PCR of the original polynucleotide with 0.1 mM $MnCl_2$ and unbalanced nucleotide concentrations. See, *e.g.*, U.S. patent application 08/337,915, filed November 10, 1994 or International application PCT/US95/14692, filed 11/10/95. The nucleic acid encoding a green fluorescent protein can be isolated by polymerase chain reaction of cDNA from *A. victoria* using primers based on the DNA sequence of *A. victoria* green fluorescent protein, as presented in Fig. 3. PCR methods are described in, for example, U.S. Pat. No. 4,683,195; Mullis et al. (1987) *Cold Spring Harbor Symp. Quant. Biol.* 51:263; and Erlich, ed., *PCR Technology*, (Stockton Press, NY, 1989).

The construction of expression vectors and the expression of genes in transfected cells involves the use of molecular cloning techniques also well known in the art. Sambrook et al., *Molecular Cloning -- A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, (1989) and *Current Protocols in Molecular Biology*, F.M. Ausubel et al., eds., (Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc.). The expression vector can be adapted for function in prokaryotes or eukaryotes by inclusion of appropriate promoters, replication sequences, markers, etc.

Nucleic acids used to transfect cells with sequences coding for expression of the polypeptide of interest generally will be in the form of an expression vector including

expression control sequences operatively linked to a nucleotide sequence coding for expression of the polypeptide. As used, the term "nucleotide sequence coding for expression of" a polypeptide refers to a sequence that, upon transcription and translation of mRNA, produces the polypeptide. This can include sequences containing, *e.g.*, introns. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus, expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (*i.e.*, ATG) in front of a protein-encoding gene, splicing signals for introns, maintenance of the correct reading frame of that gene to permit proper translation of the mRNA, and stop codons.

Methods which are well known to those skilled in the art can be used to construct expression vectors containing the fluorescent protein coding sequence and appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. (See, for example, the techniques described in Maniatis, *et al., Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y., 1989).

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the $CaCl_2$ method by procedures well known in the art. Alternatively, $MgCl_2$ or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell or by electroporation.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransfected with DNA sequences encoding the fusion polypeptide of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (*Eukaryotic Viral*

Vectors, Cold Spring Harbor Laboratory, Gluzman ed., 1982). Preferably, a eukaryotic host is utilized as the host cell as described herein.

Techniques for the isolation and purification of either microbially or eukaryotically expressed polypeptides of the invention may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies or antigen.

In one embodiment recombinant fluorescent proteins can be produced by expression of nucleic acid encoding for the protein in *E. coli*. *Aequorea*-related fluorescent proteins are best expressed by cells cultured between about 15□ C and 30□ C but higher temperatures (e.g. 37□ C) are possible. After synthesis, these enzymes are stable at higher temperatures (e.g., 37□ C) and can be used in assays at those temperatures.

A variety of host-expression vector systems may be utilized to express fluorescent protein coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing a fluorescent protein coding sequence; yeast transformed with recombinant yeast expression vectors containing the fluorescent protein coding sequence; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing a fluorescent protein coding sequence; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing a fluorescent protein coding sequence; or animal cell systems infected with recombinant virus expression vectors (*e.g.*, retroviruses, adenovirus, vaccinia virus) containing a fluorescent protein coding sequence, or transformed animal cell systems engineered for stable expression.

Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, *etc.* may be used in the expression vector (see, *e.g.*, Bitter, *et al.*, Methods in Enzymology 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage □, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. When cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the

retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the inserted fluorescent protein coding sequence.

In bacterial systems a number of expression vectors may be advantageously selected depending upon the use intended for the fluorescent protein expressed. For example, when large quantities of the fluorescent protein are to be produced, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Those which are engineered to contain a cleavage site to aid in recovering fluorescent protein are preferred.

In yeast, a number of vectors containing constitutive or inducible promoters may be used. For a review see, Current Protocols in Molecular Biology, Vol. 2, Ed. Ausubel, *et al.*, Greene Publish. Assoc. & Wiley Interscience, Ch. 13, 1988; Grant, *et al.*, Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Eds. Wu & Grossman, 31987, Acad. Press, N.Y., Vol. 153, pp.516-544, 1987; Glover, DNA Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3, 1986; and Bitter, Heterologous Gene Expression in Yeast, Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684, 1987; and The Molecular Biology of the Yeast Saccharomyces, Eds. Strathern *et al.*, Cold Spring Harbor Press, Vols. I and II, 1982. A constitutive yeast promoter such as ADH or LEU2 or an inducible promoter such as GAL may be used (Cloning in Yeast, Ch. 3, R. Rothstein In: DNA Cloning Vol.11, A Practical Approach, Ed. DM Glover, IRL Press, Wash., D.C., 1986). Alternatively, vectors may be used which promote integration of foreign DNA sequences into the yeast chromosome.

In cases where plant expression vectors are used, the expression of a fluorescent protein coding sequence may be driven by any of a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV (Brisson, *et al.*, *Nature* 310:511-514, 1984), or the coat protein promoter to TMV (Takamatsu, *et al.*, *EMBO J.* 6:307-311, 1987) may be used; alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi, *et al.*, 1984, *EMBO J.* 3:1671-1680; Broglie, *et al.*, *Science* 224:838-843, 1984); or heat shock promoters, *e.g.*, soybean hsp17.5-E or hsp17.3-B (Gurley, *et al.*, *Mol. Cell. Biol.* 6:559-565, 1986) may be used. These constructs can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation,

microinjection, electroporation, *etc.* For reviews of such techniques see, for example, Weissbach & Weissbach, *Methods for Plant Molecular Biology*, Academic Press, NY, Section VIII, pp. 421-463, 1988; and Grierson & Corey, *Plant Molecular Biology*, 2d Ed., Blackie, London, Ch. 7-9, 1988.

An alternative expression system which could be used to express fluorescent protein is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The fluorescent protein coding sequence may be cloned into non-essential regions (for example, the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the fluorescent protein coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed, see Smith, *et al., J. Viol.* 46:584, 1983; Smith, U.S. Patent No. 4,215,051.

Eukaryotic systems, and preferably mammalian expression systems, allow for proper post-translational modifications of expressed mammalian proteins to occur. Eukaryotic cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, phosphorylation, and, advantageously secretion of the gene product should be used as host cells for the expression of fluorescent protein. Such host cell lines may include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, Jurkat, HEK-293, and WI38.

Mammalian cell systems which utilize recombinant viruses or viral elements to direct expression may be engineered. For example, when using adenovirus expression vectors, the fluorescent protein coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the fluorescent protein in infected hosts (*e.g.*, see Logan & Shenk, *Proc. Natl. Acad. Sci. USA*, 81: 3655-3659, 1984). Alternatively, the vaccinia virus 7.5K promoter may be used. (*e.g.*, see, Mackett, *et al.*, *Proc. Natl. Acad. Sci. USA* ,79: 7415-7419, 1982; Mackett, *et al., J.*

Virol. 49: 857-864, 1984; Panicali, *et al.*, *Proc. Natl. Acad. Sci. USA* 79: 4927-4931, 1982). Of particular interest are vectors based on bovine papilloma virus which have the ability to replicate as extrachromosomal elements (Sarver, *et al.*, *Mol. Cell. Biol.* 1: 486, 1981). Shortly after entry of this DNA into mouse cells, the plasmid replicates to about 100 to 200 copies per cell. Transcription of the inserted cDNA does not require integration of the plasmid into the host's chromosome, thereby yielding a high level of expression. These vectors can be used for stable expression by including a selectable marker in the plasmid, such as the *neo* gene. Alternatively, the retroviral genome can be modified for use as a vector capable of introducing and directing the expression of the fluorescent protein gene in host cells (Cone & Mulligan, *Proc. Natl. Acad. Sci. USA*, 81:6349-6353, 1984). High level expression may also be achieved using inducible promoters, including, but not limited to, the metallothionine IIA promoter and heat shock promoters.

The invention can also include a localization sequence, such as a nuclear localization sequence, an endoplasmic reticulum localization sequence, a peroxisome localization sequence, a mitochondrial localization sequence, or a localized protein. Localization sequences can be targeting sequences which are described, for example, in "Protein Targeting", chapter 35 of Stryer, L., *Biochemistry* (4th ed.). W.H. Freeman, 1995. The localization sequence can also be a localized protein. Some important localization sequences include those targeting the nucleus (KKKRK), mitochondrion (amino terminal MLRTSSLFTRRVQPSLFRNILRLQST-), endoplasmic reticulum (KDEL at C-terminus, assuming a signal sequence present at N-terminus), peroxisome (SKF at C-terminus), prenylation or insertion into plasma membrane (CaaX, CC, CXC, or CCXX at C-terminus), cytoplasmic side of plasma membrane (fusion to SNAP-25), or the Golgi apparatus (fusion to furin).

For long-term, high-yield production of recombinant proteins, stable expression is preferred. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with the fluorescent protein cDNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, *etc.*), and a selectable marker. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. For example, following the introduction of foreign DNA,

engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, *et al.*, *Cell*, 11: 223, 1977), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA*, 48:2026, 1962), and adenine phosphoribosyltransferase (Lowy, *et al.*, *Cell*, 22: 817, 1980) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, *et al.*, *Proc. Natl. Acad. Sci. USA*, 77: 3567, 1980; O'Hare, *et al.*, *Proc. Natl. Acad. Sci. USA*, 8: 1527, 1981); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA*, 78: 2072, 1981; neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, *J. Mol. Biol.*, 150:1, 1981); and hygro, which confers resistance to hygromycin (Santerre, *et al.*, *Gene*, 30: 147, 1984) genes. Recently, additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. USA*, 85:8047, 1988); and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue L., In: *Current Communications in Molecular Biology*, Cold Spring Harbor Laboratory, ed., 1987).

DNA sequences encoding the fluorescence protein polypeptide of the invention can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, in other words when the foreign DNA is continuously maintained in the host, are known in the art.

The expression vector can be transfected into a host cell for expression of the recombinant nucleic acid. Host cells can be selected for high levels of expression in order to purify the fluorescent protein fusion protein. *E. coli* is useful for this purpose. Alternatively, the host cell can be a prokaryotic or eukaryotic cell selected to study the activity of an enzyme produced by the cell. In this case, the linker peptide is selected to

include an amino acid sequence recognized by the protease. The cell can be, *e.g.*, a cultured cell or a cell *in vivo*.

A primary advantage of fluorescent protein fusion proteins is that they are prepared by normal protein biosynthesis, thus completely avoiding organic synthesis and the requirement for customized unnatural amino acid analogs. The constructs can be expressed in *E. coli* in large scale for *in vitro* assays. Purification from bacteria is simplified when the sequences include polyhistidine tags for one-step purification by nickel-chelate chromatography. Alternatively, the substrates can be expressed directly in a desired host cell for assays *in situ*.

In another embodiment, the invention provides a transgenic non-human animal that expresses a nucleic acid sequence which encodes the fluorescent protein.

The "non-human animals" of the invention comprise any non-human animal having nucleic acid sequence which encodes a fluorescent protein. Such non-human animals include vertebrates such as rodents, non-human primates, sheep, dog, cow, pig, amphibians, and reptiles. Preferred non-human animals are selected from the rodent family including rat and mouse, most preferably mouse. The "transgenic non-human animals" of the invention are produced by introducing "transgenes" into the germline of the non-human animal. Embryonal target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonal target cell. The zygote is the best target for micro-injection. In the mouse, the male pronucleus reaches the size of approximately 20 micrometers in diameter which allows reproducible injection of 1-2 pl of DNA solution. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host gene before the first cleavage (Brinster *et al., Proc. Natl. Acad. Sci. USA* 82:4438-4442, 1985). As a consequence, all cells of the transgenic non-human animal will carry the incorporated transgene. This will in general also be reflected in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene. Microinjection of zygotes is the preferred method for incorporating transgenes in practicing the invention.

The term "transgenic" is used to describe an animal which includes exogenous genetic material within all of its cells. A "transgenic" animal can be produced by cross-breeding two chimeric animals which include exogenous genetic material within cells used

in reproduction. Twenty-five percent of the resulting offspring will be transgenic *i.e.*, animals which include the exogenous genetic material within all of their cells in both alleles. 50% of the resulting animals will include the exogenous genetic material within one allele and 25% will include no exogenous genetic material.

Retroviral infection can also be used to introduce transgene into a non-human animal. The developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During this time, the blastomeres can be targets for retro viral infection (Jaenich, R., *Proc. Natl. Acad. Sci USA* 73:1260-1264, 1976). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Hogan, *et al.* (1986) in Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The viral vector system used to introduce the transgene is typically a replication-defective retro virus carrying the transgene (Jahner, *et al.*, *Proc. Natl. Acad. Sci. USA* 82:6927-6931, 1985; Van der Putten, *et al.*, *Proc. Natl. Acad. Sci USA* 82:6148-6152, 1985). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten, *supra*; Stewart, *et al.*, *EMBO J.* 6:383-388, 1987). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (D. Jahner *et al.*, *Nature* 298:623-628, 1982). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic nonhuman animal. Further, the founder may contain various retro viral insertions of the transgene at different positions in the genome which generally will segregate in the offspring. In addition, it is also possible to introduce transgenes into the germ line, albeit with low efficiency, by intrauterine retro viral infection of the midgestation embryo (D. Jahner *et al.*, *supra*).

A third type of target cell for transgene introduction is the embryonal stem cell (ES). ES cells are obtained from pre-implantation embryos cultured *in vitro* and fused with embryos (M. J. Evans *et al. Nature* 292:154-156, 1981; M.O. Bradley *et al.*, *Nature* 309: 255-258, 1984; Gossler, *et al.*, *Proc. Natl. Acad. Sci USA* 83: 9065-9069, 1986; and Robertson *et al.*, *Nature* 322:445-448, 1986). Transgenes can be efficiently introduced into the ES cells by DNA transfection or by retro virus-mediated transduction. Such transformed ES cells can thereafter be combined with blastocysts from a nonhuman animal. The ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal. (For review see Jaenisch, R., *Science* 240: 1468-1474, 1988).

"Transformed" means a cell into which (or into an ancestor of which) has been introduced, by means of recombinant nucleic acid techniques, a heterologous nucleic acid molecule. "Heterologous" refers to a nucleic acid sequence that either originates from another species or is modified from either its original form or the form primarily expressed in the cell.

"Transgene" means any piece of DNA which is inserted by artifice into a cell, and becomes part of the genome of the organism (*i.e.*, either stably integrated or as a stable extrachromosomal element) which develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (*i.e.*, foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism. Included within this definition is a transgene created by the providing of an RNA sequence which is transcribed into DNA and then incorporated into the genome. The transgenes of the invention include DNA sequences which encode which encodes the fluorescent protein which may be expressed in a transgenic non-human animal. The term "transgenic" as used herein additionally includes any organism whose genome has been altered by *in vitro* manipulation of the early embryo or fertilized egg or by any transgenic technology to induce a specific gene knockout. The term "gene knockout" as used herein, refers to the targeted disruption of a gene *in vivo* with complete loss of function that has been achieved by any transgenic technology familiar to those in the art. In one embodiment, transgenic animals having gene knockouts are those in which the target gene has been rendered nonfunctional by an insertion targeted to the gene to be rendered non-functional by homologous recombination. As used herein, the term "transgenic" includes any transgenic technology familiar to those in the art which can produce an organism carrying an introduced transgene or one in which an endogenous gene has been rendered non-functional or "knocked out."

III. USES OF ENGINEERED FLUORESCENT PROTEINS

The proteins of this invention are useful in any methods that employ fluorescent proteins.

The engineered fluorescent proteins of this invention are useful as fluorescent markers in the many ways fluorescent markers already are used. This includes, for example, coupling engineered fluorescent proteins to antibodies, nucleic acids or other receptors for use in detection assays, such as immunoassays or hybridization assays.

The engineered fluorescent proteins of this invention are useful to track the movement of proteins in cells. In this embodiment, a nucleic acid molecule encoding the fluorescent protein is fused to a nucleic acid molecule encoding the protein of interest in an expression vector. Upon expression inside the cell, the protein of interest can be localized based on fluorescence. In another version, two proteins of interest are fused with two engineered fluorescent proteins having different fluorescent characteristics.

The engineered fluorescent proteins of this invention are useful in systems to detect induction of transcription. In certain embodiments, a nucleotide sequence encoding the engineered fluorescent protein is fused to expression control sequences of interest and the expression vector is transfected into a cell. Induction of the promoter can be measured by detecting the expression and/or quantity of fluorescence. Such constructs can be used used to follow signaling pathways from receptor to promoter.

The engineered fluorescent proteins of this invention are useful in applications involving FRET. Such applications can detect events as a function of the movement of fluorescent donors and acceptor towards or away from each other. One or both of the donor/acceptor pair can be a fluorescent protein. A preferred donor and receptor pair for FRET based assays is a donor with a T203I mutation and an acceptor with the mutation T203X, wherein X is an aromatic amino acid-39, especially T203Y, T203W, or T203H. In a particularly useful pair the donor contains the following mutations: S72A, K79R, Y145F, M153A and T203I (with a excitation peak of 395 nm and an emission peak of 511 nm) and the acceptor contains the following mutations S65G, S72A, K79R, and T203Y. This particular pair provides a wide separation between the excitation and emission peaks of the donor and provides good overlap between the donor emission spectrum and the acceptor excitation spectrum. Other red-shifted mutants, such as those described herein, can also be used as the acceptor in such a pair.

In one aspect, FRET is used to detect the cleavage of a substrate having the donor and acceptor coupled to the substrate on opposite sides of the cleavage site. Upon cleavage of the substrate, the donor/acceptor pair physically separate, eliminating FRET. Assays involve contacting the substrate with a sample, and determining a qualitative or quantitative change in FRET. In one embodiment, the engineered fluorescent protein is used in a substrate for □-lactamase. Examples of such substrates are described in United States patent applications 08/407,544, filed March 20, 1995 and International Application

PCT/US96/04059, filed March 20, 1996. In another embodiment, an engineered fluorescent protein donor/acceptor pair are part of a fusion protein coupled by a peptide having a proteolytic cleavage site. Such tandem fluorescent proteins are described in United States patent application 08/594,575, filed January 31, 1996.

In another aspect, FRET is used to detect changes in potential across a membrane. A donor and acceptor are placed on opposite sides of a membrane such that one translates across the membrane in response to a voltage change. This creates a measurable FRET. Such a method is described in United States patent application 08/481,977, filed June 7, 1995 and International Application PCT/US96/09652, filed June 6, 1996.

The engineered protein of this invention are useful in the creation of fluorescent substrates for protein kinases. Such substrates incorporate an amino acid sequence recognizable by protein kinases. Upon phosphorylation, the engineered fluorescent protein undergoes a change in a fluorescent property. Such substrates are useful in detecting and measuring protein kinase activity in a sample of a cell, upon transfection and expression of the substrate. Preferably, the kinase recognition site is placed within about 20 amino acids of a terminus of the engineered fluorescent protein. The kinase recognition site also can be placed in a loop domain of the protein. (See, e.g. Figure 1B.) Methods for making fluorescent substrates for protein kinases are described in United States patent application 08/680,877, filed July 16, 1996.

A protease recognition site also can be introduced into a loop domain. Upon cleavage, fluorescent property changes in a measurable fashion.

The invention also includes a method of identifying a test chemical. Typically, the method includes contacting a test chemical a sample containing a biological entity labeled with a functional, engineered fluorescent protein or a polynucleotide encoding said functional, engineered fluorescent protein. By monitoring fluorescence (i.e. a fluorescent property) from the sample containing the functional engineered fluorescent protein it can be determined whether a test chemical is active. Controls can be included to insure the specificity of the signal. Such controls include measurements of a fluorescent property in the absence of the test chemical, in the presence of a chemical with an expected activity (e.g., a known modulator) or engineered controls (e.g., absence of engineered fluorescent protein, absence of engineered fluorescent protein polynucleotide or the absence of operably linkage of the engineered fluorescent protein).

The fluorescence in the presence of a test chemical can be greater or less than in the absence of said test chemical. For instance if the engineered fluorescent protein is used a reporter of gene expression, the test chemical may up or down regulate gene expression. For such types of screening, the polynucleotide encoding the functional, engineered fluorescent protein is operatively linked to a genomic polynucleotide or a re. Alternatively, the functional, engineered fluorescent protein is fused to second functional protein. This embodiment can be used to track localization of the second protein or to track protein-protein interactions using energy transfer.

IV. PROCEDURES

Fluorescence in a sample is measured using a fluorimeter. In general, excitation radiation from an excitation source having a first wavelength, passes through excitation optics. The excitation optics cause the excitation radiation to excite the sample. In response, fluorescent proteins in the sample emit radiation which has a wavelength that is different from the excitation wavelength. Collection optics then collect the emission from the sample. The device can include a temperature controller to maintain the sample at a specific temperature while it is being scanned. According to one embodiment, a multi-axis translation stage moves a microtiter plate holding a plurality of samples in order to position different wells to be exposed. The multi-axis translation stage, temperature controller, auto-focusing feature, and electronics associated with imaging and data collection can be managed by an appropriately programmed digital computer. The computer also can

transform the data collected during the assay into another format for presentation. This process can be miniaturized and automated to enable screening many thousands of compounds.

Methods of performing assays on fluorescent materials are well known in the art and are described in, e.g., Lakowicz, J.R., *Principles of Fluorescence Spectroscopy*, New York:Plenum Press (1983); Herman, B., Resonance energy transfer microscopy, in: *Fluorescence Microscopy of Living Cells in Culture, Part B, Methods in Cell Biology*, vol. 30, ed. Taylor, D.L. & Wang, Y.-L., San Diego: Academic Press (1989), pp. 219-243; Turro, N.J., *Modern Molecular Photochemistry*, Menlo Park: Benjamin/Cummings Publishing Col, Inc. (1978), pp. 296-361.

The following examples are provided by way of illustration, not by way of limitation.

EXAMPLES

As a step in understanding the properties of GFP, and to aid in the tailoring of GFPs with altered characteristics, we have determined the three dimensional structure at 1.9 Å resolution of the S65T mutant (R. Heim et al. *Nature* 373:664-665 (1995)) of *A. victoria* GFP. This mutant also contains the ubiquitous Q80R substitution, which accidentally occurred in the early distribution of the GFP cDNA and is not known to have any effect on the protein properties (M. Chalfie et al. *Science* 263:802-805 (1994)).

Histidine-tagged S65T GFP (R. Heim et al. *Nature* 373:664-665 (1995)) was overexpressed in JM109/pRSET$_B$ in 4 l YT broth plus ampicillin at 37□, 450 rpm and 5 l/min air flow. The temperature was reduced to 25□ at A_{595} = 0.3, followed by induction with 1mM isopropylthiogalactoside for 5h. Cell paste was stored at -80□ overnight, then was resuspended in 50 mM HEPES pH 7.9, 0.3 M NaCl, 5 mM 2-mercaptoethanol, 0.1 mM phenylmethyl-sulfonylfluoride (PMSF), passed once through a French press at 10,000 psi, then centrifuged at 20 K rpm for 45 min. The supernatant was applied to a Ni-NTA-agarose column (Qiagen), followed by a wash with 20 mM imidazole, then eluted with 100 mM imidazole. Green fractions were pooled and subjected to chymotryptic (Sigma) proteolysis (1:50 w/w) for 22 h at RT. After addition of 0.5 mM PMSF, the digest was reapplied to the

Ni column. N-terminal sequencing verified the presence of the correct N-terminal methionine. After dialysis against 20 mM HEPES, pH 7.5 and concentration to $A_{490} = 20$, rod-shaped crystals were obtained at RT in hanging drops containing 5 □l protein and 5 □l well solution, 22-26% PEG 4000 (Serva), 50 mM HEPES pH 8.0-8.5, 50 mM $MgCl_2$ and 10 mM 2-mercapto-ethanol within 5 days. Crystals were 0.05 mm across and up to 1.0 mm long. The space group is $P2_12_12_1$ with a = 51.8, b = 62.8, c = 70.7 Å, Z=4. Two crystal forms of wild-type GFP, unrelated to the present form, have been described by M. A. Perrozo, K. B. Ward, R. B. Thompson, & W. W. Ward. *J. Biol. Chem.* 203, 7713-7716 (1988).

The structure of GFP was determined by multiple isomorphous replacement and anomalous scattering (Table E), solvent flattening, phase combination and crystallographic refinement. The most remarkable feature of the fold of GFP is an eleven stranded ß-barrel wrapped around a single central helix (Fig. 1A and 1B), where each strand consists of approximately 9-13 residues. The barrel forms a nearly perfect cylinder 42 Å long and 24 Å in diameter. The N-terminal half of the polypeptide comprises three anti-parallel strands, the central helix, and then 3 more anti-parallel strands, the latter of which (residues 118-123) is parallel to the N-terminal strand (residues 11-23). The polypeptide backbone then crosses the "bottom" of the molecule to form the second half of the barrel in a five-strand Greek Key motif. The top end of the cylinder is capped by three short, distorted helical segments, while one short, very distorted helical segment caps the bottom of the cylinder. The main-chain hydrogen bonding lacing the surface of the cylinder very likely accounts for the unusual stability of the protein towards denaturation and proteolysis. There are no large segments of the polypeptide that could be excised while preserving the intactness of the shell around the chromophore. Thus it would seem difficult to re-engineer GFP to reduce its molecular weight (J. Dopf & T.M. Horiagon *Gene* 173:39-43 (1996)) by a large percentage.

The *p*-hydroxybenzylideneimidazolidinone chromophore (C. W. Cody et al. *Biochemistry* 32:1212-1218 (1993)) is completely protected from bulk solvent and centrally located in the molecule. The total and presumably rigid encapsulation is probably responsible for the small Stokes' shift (i.e. wavelength difference between excitation and emission maxima), high quantum yield of fluorescence, inability of O_2 to quench the excited state (B.D. Nageswara Rao et al. *Biophys. J.* 32:630-632 (1980)), and resistance of the

chromophore to titration of the external pH (W. W. Ward. *Bioluminescence and Chemiluminescence* (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman. *Biochemistry* 21:4535-4540 (1982); W. W. Ward et al. *Photochem. Photobiol.* 35:803-808 (1982)). It also allows one to rationalize why fluorophore formation should be a spontaneous intramolecular process (R. Heim et al. *Proc. Natl. Acad. Sci.* USA 91:12501-12504 (1994)), as it is difficult to imagine how an enzyme could gain access to the substrate. The plane of the chromophore is roughly perpendicular (60□) to the symmetry axis of the surrounding barrel. One side of the chromophore faces a surprisingly large cavity, that occupies a volume of approximately 135 $Å^3$ (B. Lee & F. M. Richards. *J. Mol. Biol.* 55:379-400 (1971)). The atomic radii were those of Lee & Richards, calculated using the program MS with a probe radius of 1.4 Å. (M. L. Connolly, *Science* 221:709-713 (1983)). The cavity does not open out to bulk solvent. Four water molecules are located in the cavity, forming a chain of hydrogen bonds linking the buried side chains of Glu^{222} and Gln^{69}. Unless occupied, such a large cavity would be expected to de-stabilize the protein by several kcal/mol (S. J. Hubbard et al., *Protein Engineering* 7:613-626 (1994); A. E. Eriksson et al. *Science* 255:178-183 (1992)). Part of the volume of the cavity might be the consequence of the compaction resulting from cyclization and dehydration reactions. The cavity might also temporarily accommodate the oxidant, most likely O_2 (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995); R. Heim et al. *Proc. Natl. Acad. Sci.* USA 91:12501-12504 (1994); S. Inouye & F.I. Tsuji. *FEBS Lett.* 351:211-214 (1994)), that dehydrogenates the □-□ bond of Tyr^{66}. The chromophore, cavity, and side chains that contact the chromophore are shown in Figure 2A and a portion of the final electron density map in this vicinity in 2B.

The opposite side of the chromophore is packed against several aromatic and polar side chains. Of particular interest is the intricate network of polar interactions with the chromophore (Fig. 2C). His^{148}, Thr^{203} and Ser^{205} form hydrogen bonds with the phenolic hydroxyl; Arg^{96} and Gln^{94} interact with the carbonyl of the imidazolidinone ring and Glu^{222} forms a hydrogen bond with the side chain of Thr^{65}. Additional polar interactions, such as hydrogen bonds to Arg^{96} from the carbonyl of Thr^{62}, and the side-chain carbonyl of Gln^{183}, presumably stabilize the buried Arg^{96} in its protonated form. In turn, this buried charge suggests that a partial negative charge resides on the carbonyl oxygen of the imidazolidinone ring of the deprotonated fluorophore, as has previously been suggested (W.

W. Ward. *Bioluminescence and Chemiluminescence* (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman. *Biochemistry* 21:4535-4540 (1982); W. W. Ward et al. *Photochem. Photobiol.* 35:803-808 (1982)). Arg^{96} is likely to be essential for the formation of the fluorophore, and may help catalyze the initial ring closure. Finally, Tyr^{145} shows a typical stabilizing edge-face interaction with the benzyl ring. Trp^{57}, the only tryptophan in GFP, is located 13 Å to 15 Å from the chromophore and the long axes of the two ring systems are nearly parallel. This indicates that efficient energy transfer to the latter should occur, and explains why no separate tryptophan emission is observable (D.C. Prasher et al. *Gene* 111:229-233 (1992). The two cysteines in GFP, Cys^{48} and Cys^{70}, are 24 Å apart, too distant to form a disulfide bridge. Cys^{70} is buried, but Cys^{48} should be relatively accessible to sulfhydryl-specific reagents. Such a reagent, 5,5'-dithiobis(2-nitrobenzoic acid), is reported to label GFP and quench its fluorescence (S. Inouye & F.I. Tsuji *FEBS Lett.* 351:211-214 (1994)). This effect was attributed to the necessity for a free sulfhydryl, but could also reflect specific quenching by the 5-thio-2-nitrobenzoate moiety that would be attached to Cys^{48}.

Although the electron density map is for the most part consistent with the proposed structure of the chromophore (D.C. Prasher et al. *Gene* 111:229-233 (1992); C. W. Cody et al. *Biochemistry* 32:1212-1218 (1993)) in the *cis* [Z-] configuration, with no evidence for any substantial fraction of the opposite isomer around the chromophore double bond, difference features are found at >4 □ in the final (F_o-F_c) electron density map that can be interpreted to represent either the intact, uncyclized polypeptide or a carbinolamine (inset to Fig. 2C). This suggests that a significant fraction, perhaps as much as 30% of the molecules in the crystal, have failed to undergo the final dehydration reaction. Confirmation of incomplete dehydration comes from electrospray mass spectrometry, which consistently shows that the average masses of both wild-type and S65T GFP (31,086±4 and 31,099.5±4 Da, respectively) are 6-7 Da higher than predicted (31,079 and 31,093 Da, respectively) for the fully matured proteins. Such a discrepancy could be explained by a 30-35% mole fraction of apoprotein or carbinolamine with 18 or 20 Da higher molecular weight The natural abundance of ^{13}C and ^{2}H and the finite resolution of the Hewlett-Packard 5989B electrospray mass spectrometer used to make these measurements do not permit the individual peaks to be resolved, but instead yields an average mass peak with a full width at *half maximum* of approximately 15 Da. The molecular weights shown include the His-tag,

which has the sequence MRGSHHHHHH GMASMTGGQQM GRDLYDDDDK DPPAEF (SEQ ID NO:5). Mutants of GFP that increase the efficiency of fluorophore maturation might yield somewhat brighter preparations. In a model for the apoprotein, the Thr^{65}-Tyr^{66} peptide bond is approximately in the □-helical conformation, while the peptide of Tyr^{66}-Gly^{67} appears to be tipped almost perpendicular to the helix axis by its interaction with Arg^{96}. This further supports the speculation that Arg^{96} is important in generating the conformation required for cyclization, and possibly also for promoting the attack of Gly^{67} on the carbonyl carbon of Thr^{65} (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995)).

The results of previous random mutagenesis have implicated several amino acid side chains to have substantial effects on the spectra and the atomic model confirms that these residues are close to the chromophore. The mutations T203I and E222G have profound but opposite consequences on the absorption spectrum (T. Ehrig et al. *FEBS Letters* 367:163-166 (1995)). T203I (with wild-type Ser^{65}) lacks the 475 nm absorbance peak usually attributed to the anionic chromophore and shows only the 395 nm peak thought to reflect the neutral chromophore (R. Heim et al. *Proc. Natl. Acad. Sci.* USA 91:12501-12504 (1994); T. Ehrig et al. *FEBS Letters* 367:163-166 (1995)). Indeed, Thr^{203} is hydrogen-bonded to the phenolic oxygen of the chromophore, so replacement by Ile should hinder ionization of the phenolic oxygen. Mutation of Glu^{222} to Gly (T. Ehrig et al. *FEBS Letters* 367:163-166 (1995)) has much the same spectroscopic effect as replacing Ser^{65} by Gly, Ala, Cys, Val, or Thr, namely to suppress the 395 nm peak in favor of a peak at 470-490 nm (R. Heim et al. *Nature* 373:664-665 (1995); S. Delagrave et al. *Bio/Technology* 13:151-154 (1995)). Indeed Glu^{222} and the remnant of Thr^{65} are hydrogen-bonded to each other in the present structure, probably with the uncharged carboxyl of Glu^{222} acting as donor to the side chain oxygen of Thr^{65}. Mutations E222G, S65G, S65A, and S65V would all suppress such H-bonding. To explain why only wild-type protein has both excitation peaks, Ser^{65}, unlike Thr^{65}, may adopt a conformation in which its hydroxyl donates a hydrogen bond to and stabilizes Glu^{222} as an anion, whose charge then inhibits ionization of the chromophore. The structure also explains why some mutations seem neutral. For example, Gln^{80} is a surface residue far removed from the chromophore, which explains why its accidental and ubiquitous mutation to Arg seems to have no obvious intramolecular spectroscopic effect (M. Chalfie et al. *Science* 263:802-805 (1994)).

The development of GFP mutants with red-shifted excitation and emission

maxima is an interesting challenge in protein engineering (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995); R. Heim et al. *Nature* 373:664-665 (1995); S. Delagrave et al. *Bio/Technology* 13:151-154 (1995)). Such mutants would also be valuable for avoidance of cellular autofluorescence at short wavelengths, for simultaneous multicolor reporting of the activity of two or more cellular processes, and for exploitation of fluorescence resonance energy transfer as a signal of protein-protein interaction (R. Heim & R.Y. Tsien. *Current Biol.* 6:178-182 (1996)). Extensive attempts using random mutagenesis have shifted the emission maximum by at most 6 nm to longer wavelengths, to 514 nm (R. Heim & R.Y. Tsien. *Current Biol.* 6:178-182 (1996)); previously described "red-shifted" mutants merely suppressed the 395 nm excitation peak in favor of the 475 nm peak without any significant reddening of the 505 nm emission (S. Delagrave et al. *Bio/Technology* 13:151-154 (1995)). Because Thr^{203} is revealed to be adjacent to the phenolic end of the chromophore, we mutated it to polar aromatic residues such as His, Tyr, and Trp in the hope that the additional polarizability of their □ systems would lower the energy of the excited state of the adjacent chromophore. All three substitutions did indeed shift the emission peak to greater than 520 nm (Table F). A particularly attractive mutation was T203Y/S65G/V68L/S72A, with excitation and emission peaks at 513 and 527 nm respectively. These wavelengths are sufficiently different from previous GFP mutants to be readily distinguishable by appropriate filter sets on a fluorescence microscope. The extinction coefficient, 36,500 $M^{-1}cm^{-1}$, and quantum yield, 0.63, are almost as high as those of S65T (R. Heim et al. *Nature* 373:664-665 (1995)).

Comparison of *Aequorea* GFP with other protein pigments is instructive. Unfortunately, its closest characterized homolog, the GFP from the sea pansy *Renilla reniformis* (O. Shimomura and F.H. Johnson *J. Cell. Comp. Physiol.* 59:223 (1962); J. G. Morin and J. W. Hastings, *J. Cell. Physiol.* 77:313 (1971); H. Morise et al. *Biochemistry* 13:2656 (1974); W. W. Ward *Photochem. Photobiol. Reviews* (Smith, K. C. ed.) 4:1 (1979); W. W. Ward. *Bioluminescence and Chemiluminescence* (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman *Biochemistry* 21:4535-4540 (1982); W. W. Ward et al. *Photochem. Photobiol.* 35:803-808 (1982)), has not been sequenced or cloned, though its chromophore is derived from the same FSYG sequence as in wild-type *Aequorea* GFP (R. M. San Pietro et al. *Photochem. Photobiol.* 57:63S (1993)). The closest analog for which a three dimensional structure is

available is the photoactive yellow protein (PYP, G. E. O. Borgstahl et al. *Biochemistry* 34:6278-6287 (1995)), a 14-kDa photoreceptor from halophilic bacteria. PYP in its native dark state absorbs maximally at 446 nm and transduces light with a quantum yield of 0.64, rather closely matching wild-type GFP's long wavelength absorbance maximum near 475 nm and fluorescence quantum yield of 0.72-0.85. The fundamental chromophore in both proteins is an anionic *p*-hydroxycinnamyl group, which is covalently attached to the protein via a thioester linkage in PYP and a heterocyclic iminolactam in GFP. Both proteins stabilize the negative charge on the chromophore with the help of buried cationic arginine and neutral glutamic acid groups, Arg^{52} and Glu^{46} in PYP and Arg^{96} and Glu^{222} in GFP, though in PYP the residues are close to the oxyphenyl ring whereas in GFP they are nearer the carbonyl end of the chromophore. However, PYP has an overall □/□ fold with appropriate flexibility and signal transduction domains to enable it to mediate the cellular phototactic response, whereas GFP is a much more regular and rigid □-barrel to minimize parasitic dissipation of the excited state energy as thermal or conformational motions. GFP is an elegant example of how a visually appealing and extremely useful function, efficient fluorescence, can be spontaneously generated from a cohesive and economical protein structure.

A. Summary Of GFP Structure Determination

Data were collected at room temperature in house using either Molecular Structure Corp. R-axis II or San Diego Multiwire Systems (SDMS) detectors (Cu K□) and later at beamline X4A at the Brookhaven National Laboratory at the selenium absorption edge (□ = 0.979 Å) using image plates. Data were evaluated using the HKL package (Z. Otwinowski, in *Proceedings of the CCP4 Study Weekend: Data Collection and Processing*, L. Sawyer, N. Issacs, S. Bailey, Eds. (Science and Engineering Research Council (SERC), Daresbury Laboratory, Warrington, UK, (1991)), pp 56-62; W. Minor, XDISPLAYF (Purdue University, West Lafayette, IN, 1993)) or the SDMS software (A. J. Howard et al. *Meth. Enzymol.* 114:452-471 (1985)). Each data set was collected from a single crystal. Heavy atom soaks were 2 mM in mother liquor for 2 days. Initial electron density maps were based on three heavy atom derivatives using in-house data, then later were replaced with the synchrotron data. The EMTS difference Patterson map was solved by inspection, then used to calculate difference Fourier maps of the other derivatives. Lack of closure

refinement of the heavy atom parameters was performed using the Protein package (W. Steigemann, in Ph.D. Thesis (Technical University, Munich, 1974)). The MIR maps were much poorer than the overall figure of merit would suggest, and it was clear that the EMTS isomorphous differences dominated the phasing. The enhanced anomalous occupancy for the synchrotron data provided a partial solution to the problem. Note that the phasing power was reduced for the synchrotron data, but the figure of merit was unchanged. All experimental electron density maps were improved by solvent flattening using the program DM of the CCP4 (CCP4: *A Suite of Programs for Protein Crystallography* (SERC Daresbury Laboratory, Warrington WA4 4AD UK, 1979)) package assuming a solvent content of 38%. Phase combination was performed with PHASCO2 of the Protein package using a weight of 1.0 on the atomic model. Heavy atom parameters were subsequently improved by refinement against combined phases. Model building proceeded with FRODO and O (T. A. Jones et al. *Acta. Crystallogr. Sect. A* 47:110 (1991); T. A. Jones, in *Computational Crystallography* D. Sayre, Ed. (Oxford University Press, Oxford, 1982) pp. 303-317) and crystallographic refinement was performed with the TNT package (D. E. Tronrud et al. *Acta Cryst. A* 43:489-503 (1987)). Bond lengths and angles for the chromophore were estimated using CHEM3D (Cambridge Scientific Computing). Final refinement and model building was performed against the X4A selenomethione data set, using ($2F_o$-F_c) electron density maps. The data beyond 1.9 Å resolution have not been used at this stage. The final model contains residues 2-229 as the terminal residues are not visible in the electron density map, and the side chains of several disordered surface residues have been omitted. Density is weak for residues 156-158 and coordinates for these residues are unreliable. This disordering is consistent with previous analyses showing that residues 1 and 233-238 are dispensible but that further truncations may prevent fluorescence (J. Dopf & T.M. Horiagon. *Gene* 173:39-43 (1996)). The atomic model has been deposited in the Protein Data Bank (access code 1EMA).

Table E

Diffraction Data Statistics

Crystal	Resolution (Å)	Total obs	Unique obs	Compl. (%)[a]	Compl. (shell)[b]	Rmerge (%)[c]	Riso (%)[d]
R-axix II							
Native	2.0	51907	13582	80	69	4.1	5.8
EMTS[e]	2.6	17727	6787	87	87	5.7	20.6
SeMet	2.3	44975	10292	92	88	10.2	9.3
Multiwire							
HGI4-Se	3.0	15380	4332	84	79	7.2	28.8
X4a							
SeMet	1.8	126078	19503	80	55	9.3	9.4
EMTS	2.3	57812	9204	82	66	7.2	26.3

Phasing Statistics

Derivative	Resolution (Å)	Number of sites	Phasing power[f]	Phasing Power(shell)	FOM[g]	FOM (shell)
In House						
EMTS	3.0	2	2.08	2.08	0.77	.072
SeMet	3.0	4	1.66	1.28	-	-
HGI4-Se	3.0	9	1.77	1.90	-	-
X4a						
EMTS	3.0	2	1.36	1.26	0.77	.072
SeMet	3.0	4	1.31	1.08	-	-

Atomic Model Statistics

Protein atoms	1790
Solvent atoms	94
Resol. range (Å)	20-1.9
Number of reflections (F > 0)	17676
Completeness	84.
R. factor[h]	0.175
Mean B-value (Å^2)	24.1
Deviations from ideality	
Bond lengths (Å)	0.014
Bond angles (□)	1.9
Restrained B-values (Å^2)	4.3
Ramachandran outliers	0

Notes:

(a) Completeness is the ratio of observed reflections to theoretically possible expressed as a percentage.

(b) Shell indicates the highest resolution shell, typically 0.1-0.4 Å wide.

(c) Rmerge = □ |I - <I>| / □ I, where <I> is the mean of individual observations of intensities I.

(d) Riso = □ $|I_{DER} - I_{NAT}|$ / □ I_{NAT}

(e) Derivatives were EMTS=ethymercurithiosalicylate (residues modified Cys^{48} and Cys^{70}), SeMet=selenomethionine substituted protein (Met^{1} and Met^{233} could not be located); HgI_4-SeMet = double derivative HgI_4 on SeMet background.

(f) Phasing power = $\langle F_H \rangle / \langle E \rangle$ where $\langle F_H \rangle$=r.m.s. heavy atom scattering and <E>=lack of closure.

(g) FOM, mean figure of merit

(h) Standard crystallographic R-factor, R = □ $||F_{obs}| - |F_{calc}||$ / □ $|F_{obs}|$

B. Spectral properties of Thr^{203} ("T203") mutants compared to S65T

The mutations F64L, V68L and S72A improve the folding of GFP at 37□ (B. P. Cormack et al. *Gene* 173:33 (1996)) but do not significantly shift the emission spectra.

TABLE F

Clone	Mutations	Excitation max.(nm)	Extinction coefficient ($10^3 M^{-1}cm^{-1}$)	Emission max.(nm)
S65T	S65T	489	39.2	511
5B	T203H/S65T	512	19.4	524
6C	T203Y/S65T	513	14.5	525
10B	T203Y/F64L/S65G/S72A	513	30.8	525
10C	T203Y/F65G/V68L/S72A	513	36.5	527
11	T203W/S65G/S72A	502	33.0	512

12H	T203Y/S65G/S72A	513	36.5	527
20A	T203Y/S65G/V68L/Q69K/S72A	515	46.0	527

The present invention provides novel long wavelength engineered fluorescent proteins. While specific examples have been provided, the above description is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification. The scope of the invention should, therefore, be determined not with reference to the above description, but instead should be determined with reference to the appended claims along with their full scope of equivalents.

All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication or patent document were so individually denoted.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The Regents of the University of California et al.

(ii) TITLE OF INVENTION: LONG WAVELENGTH MUTANT FLUORESCENT PROTEINS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fish & Richardson P.C.
(B) STREET: 4225 Executive Square, Suite 1400
(C) CITY: La Jolla
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 92037

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/024,050
(B) FILING DATE: 16-AUG-1996
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/706,408
(B) FILING DATE: 30-AUG-1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Haile, Lisa A.
(B) REGISTRATION NUMBER: 38,347
(C) REFERENCE/DOCKET NUMBER: 07257/056WO1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 619/678-5070
(B) TELEFAX: 619/678-5099

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 716 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..714

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATG AGT AAA GGA GAA GAA CTT TTC ACT GCA GTT GTC CCA ATT CTT GTT        48
Met Ser Lys Gly Glu Glu Leu Phe Thr Ala Val Val Pro Ile Leu Val
 1               5                  10                  15

GAA TTA GAT GGT GAT GTT AAT GGG CAC AAA TTT TCT GTC AGT GGA GAG        96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20                  25                  30

GGT GAA GGT GAT GTA ACA TAC GGA AAA CTT ACC CTT AAA TTT ATT TGC       144
Gly Glu Gly Asp Val Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35                  40                  45

ACT ACT GGA AAA CTA CCT GTT CCA TGG CCA ACA CTT GTC ACT ACT TTC       192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50                  55                  60

TCT TAT GGT GTT CAA TGC TTT TCA AGA TAC CCA GAT CAT ATG AAA CGG       240
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65                  70                  75                  80

CAT GAC TTT TTC AAG AGT GCC ATG CCC GAA GGT TAT GTA CAG CAA AGA       288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Gln Arg
                85                  90                  95

ACT ATA TTT TTC AAA GAT GAC GGG AAC TAC AAG ACA CGT GCT GAA GTC       336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110

AAG TTT GAA GGT GAT ACC CTT GTT AAT AGA ATC GAG TTA AAA GGT ATT       384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125

GAT TTT AAA GAA GAT GGA AAC ATT CTT GGA CAT AAA TTG GAA TAC AAC       432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

TAT AAC TCA CAC AAT GTA TAC ATC ATG GCA GAC AAA CAA AAG AAT GGA       480
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

ATC AAA GTT AAC TTC AAA ATT AGA CAC AAC ATT GAA GAT GGA AGC GTT       528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175

CAA CTA GCA GAC TAT TAT CAA CAA AAT ACT CCA ATT CTC GAT GGC CCT       576
Gln Leu Ala Asp Tyr Tyr Gln Gln Asn Thr Pro Ile Leu Asp Gly Pro
            180                 185                 190

GTC CTT TTA CCA GAC AAC CAT TAC CTG TCC ACA CAA TCT GCC CTT TCG       624
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

AAA GAT CCC AAC GAA AAG AGA GAC CAC ATG GTC CTT CTT GAG TTT GTA       672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

ACA GCT GCT GGG ATT ACA CAT GGC ATG GAT GAA CTA TAC AAA               714
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235

TA                                                                   716
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 238 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ser Lys Gly Glu Glu Leu Phe Thr Ala Val Val Pro Ile Leu Val
 1               5                  10                  15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20                  25                  30

Gly Glu Gly Asp Val Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35                  40                  45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50                  55                  60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65                  70                  75                  80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Gln Arg
                85                  90                  95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175

Gln Leu Ala Asp Tyr Tyr Gln Gln Asn Thr Pro Ile Leu Asp Gly Pro
            180                 185                 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 720 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..720

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG GTG AGC AAG GGC GAG GAG CTG TTC ACC GGG GTG GTG CCC ATC CTG        48
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
    240                 245                 250

GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG TCC GGC        96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
255                 260                 265                 270

GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC       144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
                275                 280                 285

TGC ACC ACC GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC       192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
            290                 295                 300

TTC GGC TAC GGC GTG CAG TGC TTC GCC CGC TAC CCC GAC CAC ATG AAG       240
Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys
        305                 310                 315

CAG CAG GAC TTC TTC AAG TCC GCC ATG CCC GAA GGC TAC GTC CAG GAG       288
Gln Gln Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
    320                 325                 330

CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG       336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
335                 340                 345                 350

GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG GGC       384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
                355                 360                 365

ATC GAC TTC AAG GAC GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC       432
Ile Asp Phe Lys Asp Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
            370                 375                 380

AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC       480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
        385                 390                 395

GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC       528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
    400                 405                 410

GTG CAG CCC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC       576
Val Gln Pro Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
415                 420                 425                 430

CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG AGC TAC CAG TCC GCC CTG       624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu
                435                 440                 445

AGC AAA GAC CCC AAC GAG AAG CGC GAT CAC ATG GTC CTG CTG GAG TTC       672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
            450                 455                 460

GTG ACC GCC GCC GGG ATC ACT CAC GGC ATG GAC GAG CTG TAC AAG TAA       720
Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys  *
        465                 470                 475
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 240 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1               5                  10                  15

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            20                  25                  30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                  40                  45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
    50                  55                  60

Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys
65                  70                  75                  80

Gln Gln Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125

Ile Asp Phe Lys Asp Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                 135                 140

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165                 170                 175

Val Gln Pro Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180                 185                 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu
        195                 200                 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                 215                 220

Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys  *
225                 230                 235                 240
```

WHAT IS CLAIMED IS:

1. A nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

2. The nucleic acid molecule of claim 1 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

3. The nucleic acid molecule of claim 1 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

4. The nucleic acid molecule of claim 1 or 2 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

5. The nucleic acid molecule of claim 1 or 2 wherein the amino acid sequence further comprises a mutation from Table A.

6. The nucleic acid molecule of claim 1 or 2 wherein the amino acid sequence further comprises a folding mutation.

7. The nucleic acid molecule of any of claims 1-3 wherein the nucleotide sequence encoding the protein differs from the nucleotide sequence of SEQ ID NO:1 by the substitution of at least one codon by a preferred mammalian codon.

8. The nucleic acid molecule of any of claims 1-3 encoding a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

9. An expression vector comprising expression control sequences operatively linked to a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

10. The expression vector of claim 9 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

11. The expression vector of claim 9 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y, S65G/S72A/T203Y; or S65G/S72A/T203W.

12. The expression vector of claim 10 or 11 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

13. The expression vector of claim 10 or 11 wherein the amino acid sequence further comprises a mutation from Table A.

14. The expression vector of claim 9 or 10 wherein the amino acid sequence further comprises a folding mutation.

15. The expression vector of any of claims 9-11 wherein the nucleotide sequence encoding the protein differs from the nucleotide sequence of SEQ ID NO:1 by the substitution of at least one codon by a preferred mammalian codon.

16. The expression vector of any of claims 9-11 encoding a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

17. A recombinant host cell comprising an expression vector that comprises expression control sequences operatively linked to a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

18. The recombinant host cell of claim 17 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

19. The recombinant host cell of claim 17 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

20. The recombinant host cell of claim 17 or 18 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

21. The recombinant host cell of claim 17 or 18 wherein the amino acid sequence further comprises a mutation from Table A.

22. The recombinant host cell of claim 17 or 18 wherein the amino acid sequence further comprises a folding mutation.

23. The recombinant host cell of any of claims 17-19 wherein the nucleotide sequence encoding the protein differs from the nucleotide sequence of SEQ ID NO:1 by the substitution of at least one codon by a preferred mammalian codon.

24. The recombinant host cell of any of claims 17-19 encoding a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

25. The recombinant host cell of any of claims 17-19 which is a prokaryotic cell.

26. The recombinant host cell of any of claims 17-19 which is a eukaryotic cell.

27. A functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

28. The protein of claim 27 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

29. The protein of claim 27 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

30. The protein of claim 27 or 28 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

31. The protein of claim 27 or 28 wherein the amino acid sequence further comprises a folding mutation.

32. The protein of any of claims 27-29 which is a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

33. A fluorescently labelled antibody comprising an antibody coupled to a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

34. The fluorescently labelled antibody of claim 33 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

35. The fluorescently labelled antibody of claim 33 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

36. The fluorescently labelled antibody of claim 33 or 34 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

37. The fluorescently labelled antibody of any of claims 33-35 which is a fusion protein wherein the fusion protein comprises the antibody fused to the functional engineered fluorescent protein.

38. A nucleic acid molecule comprising a nucleotide sequence encoding an antibody fused to a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

39. The nucleic acid molecule of claim 38 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

40. The nucleic acid molecule of claim 38 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

41. The nucleic acid molecule of claim 38 or 39 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

42. A fluorescently labelled nucleic acid probe comprising a nucleic acid probe coupled to a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

43. The fluorescently labelled nucleic acid probe of claim 42 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

44. The fluorescently labelled nucleic acid probe of claim 42 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

45. The nucleic acid molecule of claim 42 or 43 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

46. A nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

47. The nucleic acid molecule of claim 46 wherein the amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,

V61X, wherein X is selected from F, Y, H and C,

T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,

V68X, wherein X is selected from F, Y and H,

Q69X, wherein X is selected from K, R, E and G,

Q94X, wherein X is selected from D, E, H, K and N,

N121X, wherein X is selected from F, H, W and Y,
Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,
H148X, wherein X is selected from F, Y, N, K, Q and R,
V150X, wherein X is selected from F, Y and H,
F165X, wherein X is selected from H, Q, W and Y,
I167X, wherein X is selected from F, Y and H,
Q183X, wherein X is selected from H, Y, E and K,
N185X, wherein X is selected from D, E, H, K and Q,
L220X, wherein X is selected from H, N, Q and T,
E222X, wherein X is selected from N and Q or
V224X, wherein X is selected from H, N, Q, T, F, W and Y.

48. An expression vector comprising expression control sequences operatively linked to a nucleic acid molecule of comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

49. The expression vector of claim 48 wherein the amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,
V61X, wherein X is selected from F, Y, H and C,
T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,
V68X, wherein X is selected from F, Y and H,
Q69X, wherein X is selected from K, R, E and G,
Q94X, wherein X is selected from D, E, H, K and N,

N121X, wherein X is selected from F, H, W and Y,
Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,
H148X, wherein X is selected from F, Y, N, K, Q and R,
V150X, wherein X is selected from F, Y and H,
F165X, wherein X is selected from H, Q, W and Y,
I167X, wherein X is selected from F, Y and H,
Q183X, wherein X is selected from H, Y, E and K,
N185X, wherein X is selected from D, E, H, K and Q,
L220X, wherein X is selected from H, N, Q and T,
E222X, wherein X is selected from N and Q or
V224X, wherein X is selected from H, N, Q, T, F, W and Y.

50. A recombinant host cell comprising an expression vector that comprises expression control sequences operatively linked to a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

51. The recombinant host cell of claim 50 wherein the amino acid substitution is:
L42X, wherein X is selected from C, F, H, W and Y,
V61X, wherein X is selected from F, Y, H and C,
T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,
V68X, wherein X is selected from F, Y and H,
Q69X, wherein X is selected from K, R, E and G,

Q94X, wherein X is selected from D, E, H, K and N,

N121X, wherein X is selected from F, H, W and Y,

Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,

H148X, wherein X is selected from F, Y, N, K, Q and R,

V150X, wherein X is selected from F, Y and H,

F165X, wherein X is selected from H, Q, W and Y,

I167X, wherein X is selected from F, Y and H,

Q183X, wherein X is selected from H, Y, E and K,

N185X, wherein X is selected from D, E, H, K and Q,

L220X, wherein X is selected from H, N, Q and T,

E222X, wherein X is selected from N and Q or

V224X, wherein X is selected from H, N, Q, T, F, W and Y.

52. A functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

53. The functional engineered fluorescent protein of claim 52 wherein the amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,

V61X, wherein X is selected from F, Y, H and C,

T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,

V68X, wherein X is selected from F, Y and H,

Q69X, wherein X is selected from K, R, E and G,

Q94X, wherein X is selected from D, E, H, K and N,

N121X, wherein X is selected from F, H, W and Y,
Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,
H148X, wherein X is selected from F, Y, N, K, Q and R,
V150X, wherein X is selected from F, Y and H,
F165X, wherein X is selected from H, Q, W and Y,
I167X, wherein X is selected from F, Y and H,
Q183X, wherein X is selected from H, Y, E and K,
N185X, wherein X is selected from D, E, H, K and Q,
L220X, wherein X is selected from H, N, Q and T,
E222X, wherein X is selected from N and Q or
V224X, wherein X is selected from H, N, Q, T, F, W and Y.

54. A fluorescently labelled antibody comprising an antibody coupled to a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

55. The antibody of claim 54 wherein the amino acid substitution is:
L42X, wherein X is selected from C, F, H, W and Y,
V61X, wherein X is selected from F, Y, H and C,
T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,
V68X, wherein X is selected from F, Y and H,
Q69X, wherein X is selected from K, R, E and G,
Q94X, wherein X is selected from D, E, H, K and N,
N121X, wherein X is selected from F, H, W and Y,

Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,
H148X, wherein X is selected from F, Y, N, K, Q and R,
V150X, wherein X is selected from F, Y and H,
F165X, wherein X is selected from H, Q, W and Y,
I167X, wherein X is selected from F, Y and H,
Q183X, wherein X is selected from H, Y, E and K,
N185X, wherein X is selected from D, E, H, K and Q,
L220X, wherein X is selected from H, N, Q and T,
E222X, wherein X is selected from N and Q or
V224X, wherein X is selected from H, N, Q, T, F, W and Y.

56. A nucleic acid molecule comprising a nucleotide sequence encoding an antibody fused to a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

57. The nucleic acid molecule of claim 56 wherein the amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,
V61X, wherein X is selected from F, Y, H and C,
T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,
V68X, wherein X is selected from F, Y and H,
Q69X, wherein X is selected from K, R, E and G,
Q94X, wherein X is selected from D, E, H, K and N,
N121X, wherein X is selected from F, H, W and Y,

Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,

H148X, wherein X is selected from F, Y, N, K, Q and R,

V150X, wherein X is selected from F, Y and H,

F165X, wherein X is selected from H, Q, W and Y,

I167X, wherein X is selected from F, Y and H,

Q183X, wherein X is selected from H, Y, E and K,

N185X, wherein X is selected from D, E, H, K and Q,

L220X, wherein X is selected from H, N, Q and T,

E222X, wherein X is selected from N and Q or

V224X, wherein X is selected from H, N, Q, T, F, W and Y.

58. A fluorescently labelled nucleic acid probe comprising a nucleic acid probe coupled to a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

59. The probe of claim 58 wherein the amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,

V61X, wherein X is selected from F, Y, H and C,

T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,

V68X, wherein X is selected from F, Y and H,

Q69X, wherein X is selected from K, R, E and G,

Q94X, wherein X is selected from D, E, H, K and N,

N121X, wherein X is selected from F, H, W and Y,

Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,

H148X, wherein X is selected from F, Y, N, K, Q and R,

V150X, wherein X is selected from F, Y and H,

F165X, wherein X is selected from H, Q, W and Y,

I167X, wherein X is selected from F, Y and H,

Q183X, wherein X is selected from H, Y, E and K,

N185X, wherein X is selected from D, E, H, K and Q,

L220X, wherein X is selected from H, N, Q and T,

E222X, wherein X is selected from N and Q or

V224X, wherein X is selected from H, N, Q, T, F, W and Y.

60. A method for determining whether a mixture contains a target comprising:

contacting the mixture with a fluorescently labelled probe comprising a probe and a functional engineered fluorescent protein of claim 27 or claim 52; and

determining whether the target has bound to the probe.

61. The method of any of claim 60 the target is bound to a solid matrix.

62. A method for engineering a functional engineered fluorescent protein having a fluorescent property different than *Aequorea* green fluorescent protein, comprising substituting an amino acid that is located no more than 0.5 nm from any atom in the chromophore of an *Aequorea*-related green fluorescent protein with another amino acid; whereby the substitution alters a fluorescent property of the protein.

63. The method of claim 62 wherein the amino acid substitution alters the electronic environment of the chromophore.

64. A method for engineering a functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein comprising substituting amino acids in a loop domain of an *Aequorea*-related green fluorescent protein with amino acids so as to create a consensus sequence for phosphorylation or for proteolysis.

65. A method for producing fluorescence resonance energy transfer comprising:

providing a donor molecule comprising a functional engineered fluorescent protein of claim 27 or claim 52;

providing an appropriate acceptor molecule for the fluorescent protein; and

bringing the donor molecule and the acceptor molecule into sufficiently close contact to allow fluorescence resonance energy transfer.

66. A method for producing fluorescence resonance energy transfer comprising:

providing an acceptor molecule comprising a functional engineered fluorescent protein of claim 27 or claim 52;

providing an appropriate donor molecule for the fluorescent protein; and

bringing the donor molecule and the acceptor molecule into sufficiently close contact to allow fluorescence resonance energy transfer.

67. The method of claim 66 wherein the donor molecule is a engineered fluorescent protein whose amino acid sequence comprises the substitution T203I and the acceptor molecule is a nutant fluorescent protein whose amino acid sequence comprises the substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

68. A nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution located no more than about 0.5 nm from the chromophore of the engineered fluorescent protein, wherein the substitution alters the electronic environment of the chromophore, whereby the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

69. An expression vector comprising expression control sequences operatively linked to a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution located no more than about 0.5 nm from the chromophore of the engineered fluorescent protein, wherein the substitution alters the electronic environment of the chromophore, whereby the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

70. A functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution located no more than about 0.5 nm from the chromophore of the engineered fluorescent protein, wherein the substitution alters the electronic environment of the chromophore, whereby the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

71. A crystal of a protein comprising a fluorescent protein with an amino acid sequence substantially identical to SEQ ID NO: 2, wherein said crystal diffracts with at least a 2.0 to 3.0 angstrom resolution.

72. The crystal of claim 71, wherein the fluorescent protein has at least 200 amino acids, a completeness value of at least 80% and has a crystal stability within 0.5% of its unit cell dimensions.

73. The crystal of claim 71, wherein the amino acid sequence comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

74. The crystal of claim 71, wherein said crystal has the following unit cell dimensions in angstroms: a = 51.8, b= 62.8 and c= 70.7 with a space group of P2 2 2 and an □ angle of 90.00□, a □ angle of 90.00□ and a □ angle of 90.00□ and the crystal has a diffraction limit where 90% or greater of the potential reflections can be used to determine the coordinates of the atoms.

75. A computational method of designing a fluoresent protein comprising:

determining from a three dimensional model of a crystallized fluorescent protein comprising a fluorescent protein with a bound ligand, at least one interacting amino acid of the fluorescent protein that interacts with at least one first chemical moiety of the ligand, and

selecting at least one chemical modification of the first chemical moiety to produce a second chemical moiety with a structure to either decrease or increase an interaction between the interacting amino acid and the second chemical moiety compared to the interaction between the interacting amino acid and the first chemical moiety.

76. The computational method of claim 75, further comprising generating the three dimensional model of the crystallized protein comprising a fluorescent protein with an amino acid sequence substantially identical to SEQ ID NO:2.

77. The computational method of claim 75, wherein the selecting selects the first chemical moiety that interacts with at least one of the amino acids listed in Figs. 5-1 to 5-28.

78. The computational method of claim 75, wherein the chemical modification enhances hydrogen bonding interaction, charge interaction, hydrophobic interaction, Van Der Waals interaction or dipole interaction between the second chemical moiety and the interacting amino acid compared to the first chemical moiety and the interacting amino acid.

79. A computational method of modeling the three dimensional structure of a fluorescent protein comprising determining a three dimensional relationship between at least two atoms listed in the atomic coordinates of Figs. 5-1 to 5-28.

80. The computational method of claim 79, wherein the determining comprises determining the three dimensional structure of a fluorescent protein with an amino acid sequence at least 80% identical to SEQ ID NO:2.

81. The computational method of claim 79, wherein the determining comprises determining the three dimensional structure of a fluorescent protein with an amino acid sequence at least 95% identical to SEQ ID NO:2.

82. The computational method of claim 79, wherein the determining comprises determining the three dimensional relationship of at least 1500 atoms listed in Figs. 5-1 to 5-28.

83. A device comprising a storage device and, stored in the device, at least 10 atomic coordinates selected from the atomic coordinates listed in Figs. 5-1 to 5-28.

84. The device of claim 83, wherein the storage device is a computer readable device that stores code that receives as input the atomic coordinates.

85. The device of claim 84, wherein computer readable device is a floppy disk or a hard drive.

86. A nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at Q69, wherein said functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

87. The nucleic acid molecule of claim 86, wherein said substitution at Q69 is selected from the group of K, R, E and G.

88. The nucleic acid molecule of claim 86, wherein said amino acid sequence further comprises a function mutation at S65.

89. A nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at E222, but not including E222G, wherein said functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

90. The nucleic acid molecule of claim 89, wherein said substitution at E222 is selected from the group of N and Q.

91. The nucleic acid molecule of claim 89, wherein said amino acid sequence further comprises a function mutation at F64.

92. A nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at Y145, wherein said functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

93. The nucleic acid molecule of claim 92, wherein said substitution at Y145 is selected from the group of W, C, F, L, E, H, K and Q .

94. The nucleic acid molecule of claim 92, wherein said amino acid sequence further comprises a function mutation at Y66.

95. A method of identifying a test chemical, comprising:

contacting a test chemical a sample containing a biological entity labeled with a functional, engineered fluorescent protein or a polynucleotide encoding said functional, engineered fluorescent protein, and

detecting fluorescence of said functional engineered fluorescent protein.

96. The method of claim 95, wherein said fluorescence in the presence of a test chemical is greater than in the absence of said test chemical.

97. The method of claim 96, wherein said polynucleotide encoding said functional, engineered fluorescent protein is operatively linked to a genomic polynucleotide.

98. The method of claim 95, wherein said functional, engineered fluorescent protein is fused to second functional protein.

99. The method of claim 96, wherein said polynucleotide encoding said functional, engineered fluorescent protein is operatively linked to a response element.

100. The method of claim 96, wherein said polynucleotide encoding said functional, engineered fluorescent protein is operatively linked to a response element in a mammalian cell.

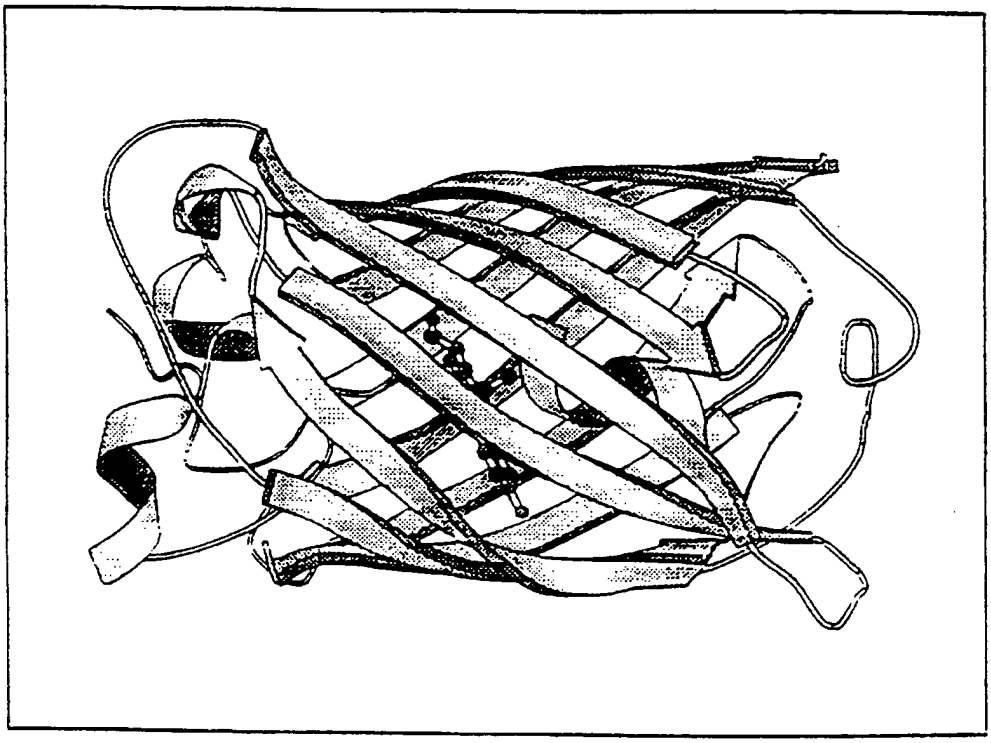


Figure 1a

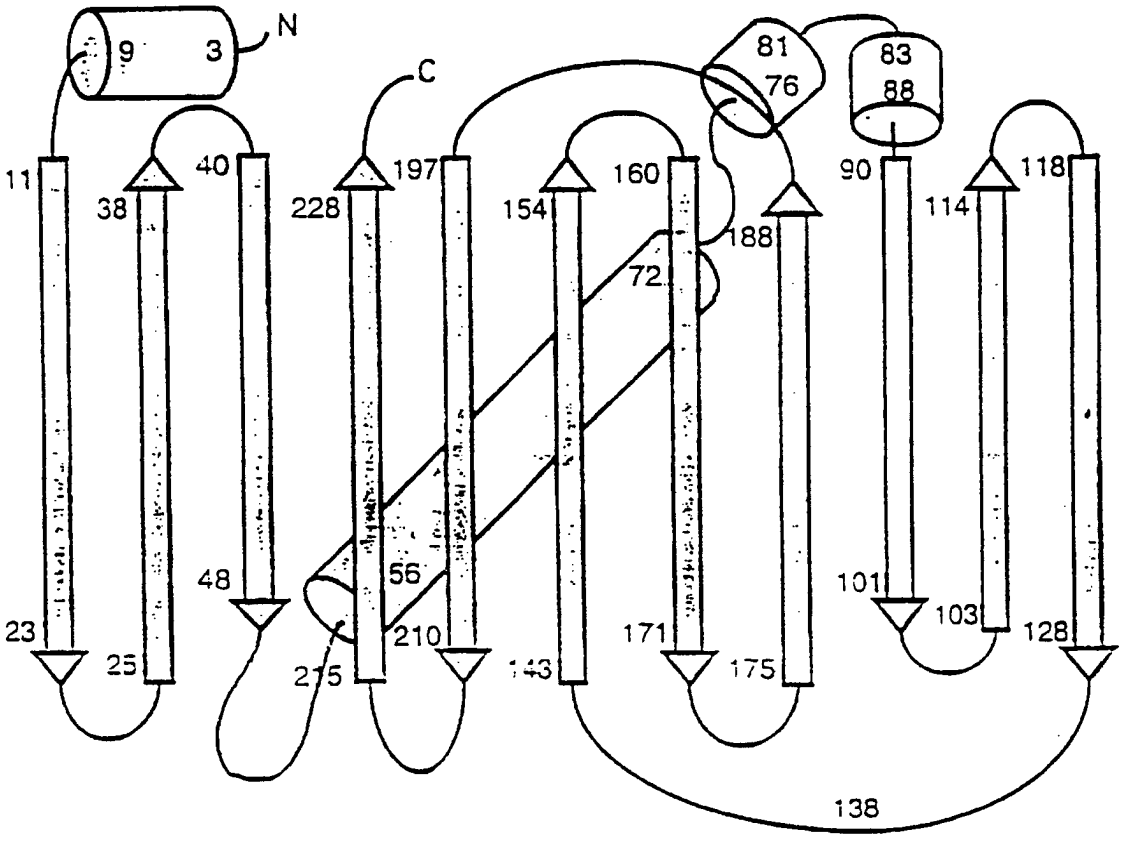

N
C
9
3
11
38
40
48
23
25
228
197
56
210
215
154
160
72
188
143
171
175
81
76
83
88
90
114
118
101
103
128
138

Figure 1b

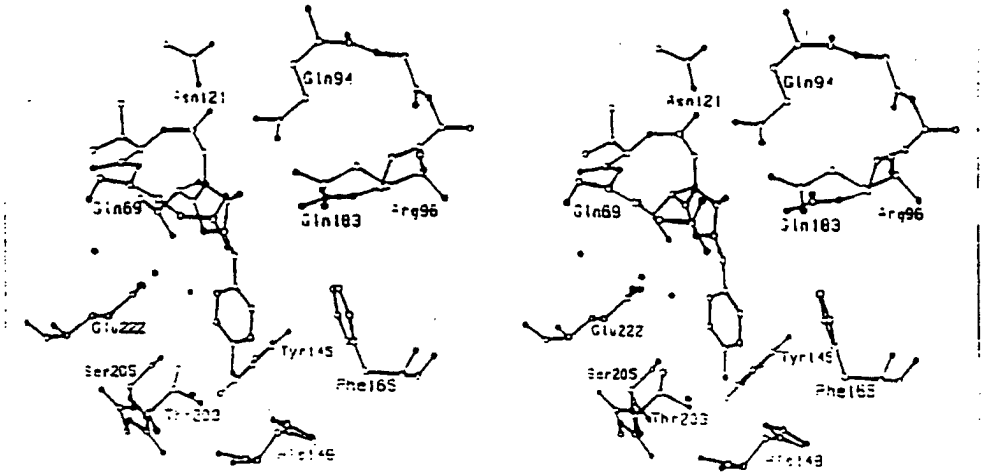

Asn121
Gln94
Gln69
Gln183
Arg96
Glu222
Ser205
Tyr145
Phe165
Asn121
Gln94
Gln69
Gln183
Arg96
Glu222
Ser205
Tyr145
Phe165

Figure 2a

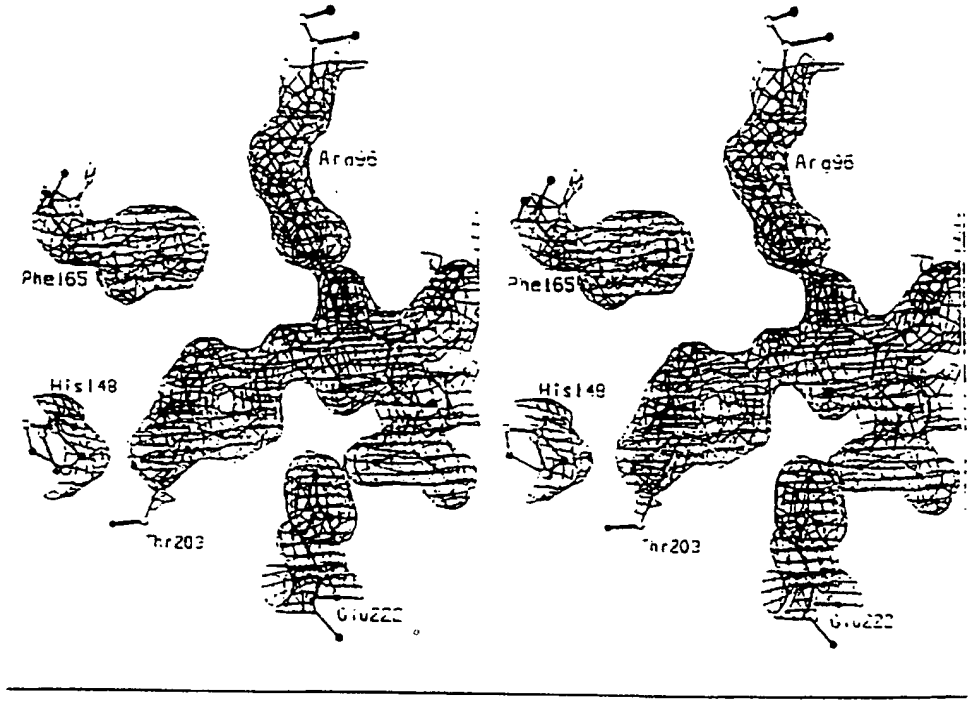

Arg96
Phe165
His148
Thr203
Glu222
Arg96
Phe165
His148
Thr203

Figure 2b

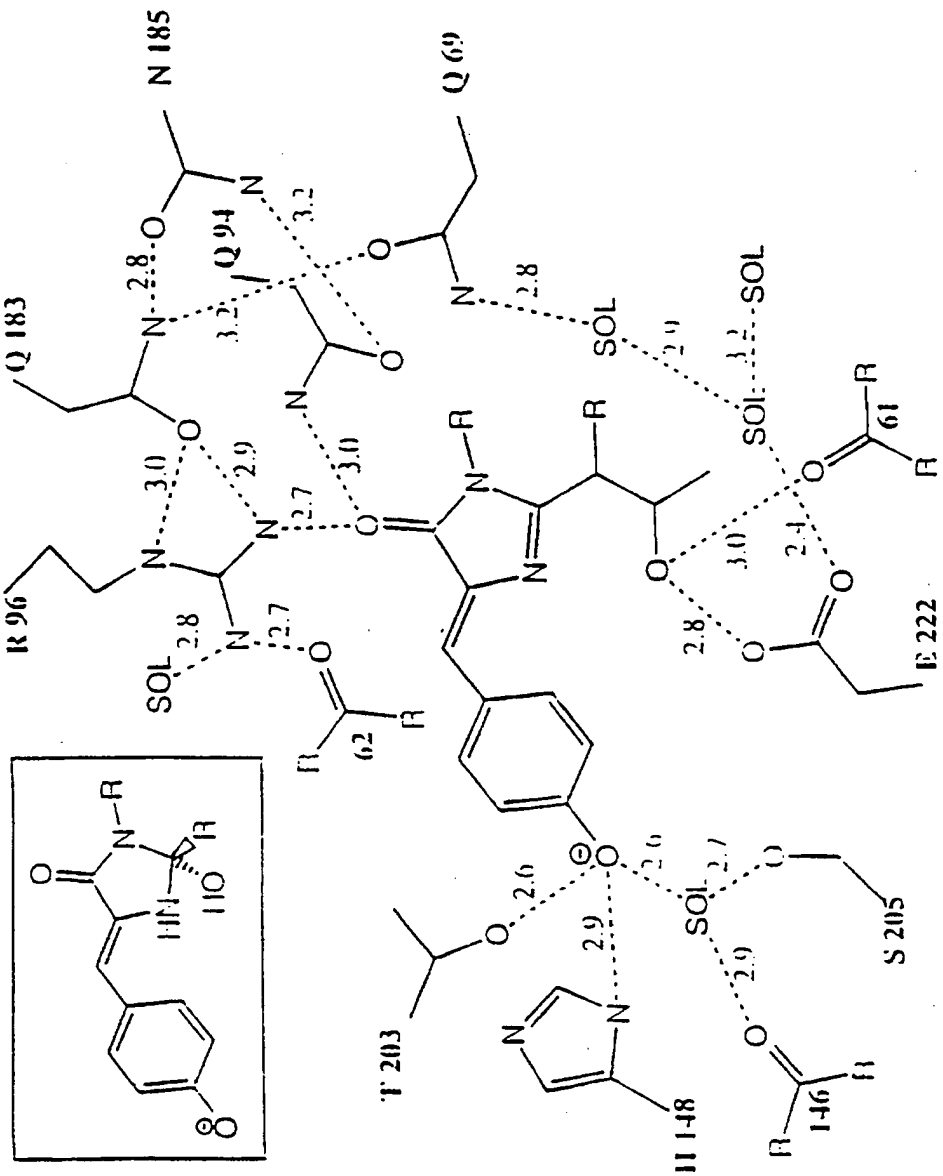


Figure 2c

(xi) SEQUENCE DESCRIPTION:

SEQ ID NO:1:
SEQ ID NO:2:

```
ATG AGT AAA GGA GAA GAA CTT TTC ACT GGA GTT GTC CCA ATT CTT GTT          48
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
 1               5                  10                  15

GAA TTA GAT GGT GAT GTT AAT GGG CAC AAA TTT TCT GTC AGT GGA GAG          96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20                  25                  30

GGT GAA GGT GAT GCA ACA TAC GGA AAA CTT ACC CTT AAA TTT ATT TGC         144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35                  40                  45

ACT ACT GGA AAA CTA CCT GTT CCA TGG CCA ACA CTT GTC ACT ACT TTC         192

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50                  55                  60

TCT TAT GGT GTT CAA TGC TTT TCA AGA TAC CCA GAT CAT ATG AAA CGG         240
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65                  70                  75                  80

CAT GAC TTT TTC AAG AGT GCC ATG CCC GAA GGT TAT GTA CAG GAA AGA         288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                85                  90                  95

ACT ATA TTT TTC AAA GAT GAC GGG AAC TAC AAG ACA CGT GCT GAA GTC         336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110

AAG TTT GAA GGT GAT ACC CTT GTT AAT AGA ATC GAG TTA AAA GGT ATT         384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125

GAT TTT AAA GAA GAT GGA AAC ATT CTT GGA CAC AAA TTG GAA TAC AAC         432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

TAT AAC TCA CAC AAT GTA TAC ATC ATG GCA GAC AAA CAA AAG AAT GGA         480
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

ATC AAA GTT AAC TTC AAA ATT AGA CAC AAC ATT GAA GAT GGA AGC GTT         528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175

CAA CTA GCA GAC CAT TAT CAA CAA AAT ACT CCA ATT GGC GAT GGC CCT         576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180                 185                 190

GTC CTT TTA CCA GAC AAC CAT TAC CTG TCC ACA CAA TCT GCC CTT TCG         624
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

AAA GAT CCC AAC GAA AAG AGA GAC CAC ATG GTC CTT CTT GAG TTT GTA         672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

ACA GCT GCT GGG ATT ACA CAT GGC ATG GAT GAA CTA TAC AAA TA              717
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
```

Figure 3

TCEDV 3553 3713A humanized codon usage. With an additional amino acid after the start met to provide optimal Kozak sequence

```
          9          18          27          36          45          54
ATG GTG AGC AAG GGC GAG GAG CTG TTC ACC GGG GTG GTG CCC ATC CTG GTC GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu

         63          72          81          90          99         108
CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG TCC GGC GAG GGC GAG GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly

        117         126         135         144         153         162
GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC TGC ACC ACC GGC AAG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu

        171         180         189         198         207         216
CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC TTC GGC TAC GGC GTG CAG TGC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Gly Tyr Gly Val Gln Cys Phe

        225         234         243         252         261         270
GCC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC TTC TTC AAG TCC GCC ATG CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro

        279         288         297         306         315         324
GAA GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys

        333         342         351         360         369         378
ACC CGC GCC GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu

        387         396         405         414         423         432
AAG GGC ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr

        441         450         459         468         477         486
AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC GGC ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile

        495         504         513         522         531         540
AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC GTG CAG CTC GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala

        549         558         567         576         585         594
GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC CCC GTG CTG CTG CCC GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp

        603         612         621         630         639         648
AAC CAC TAC CTG AGC TAC CAG TCC GCC CTG AGC AAA GAC CCC AAC GAG AAG CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg

        657         666         675         684         693         702
GAT CAC ATG GTC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT CAC GGC ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met

                711
GAC GAG CTG TAC AAG TAA 3'
--- --- --- --- ---
Asp Glu Leu Tyr Lys ***
```

Figure 4

FIG 5-1

```
CRYST1   51.767   62.845   70.666  90.00  90.00  90.00
ORIGX1      1.000000  0.000000  0.000000        0.00000
ORIGX2      0.000000  1.000000  0.000000        0.00000
ORIGX3      0.000000  0.000000  1.000000        0.00000
SCALE1      0.019317  0.000000  0.000000        0.00000
SCALE2      0.000000  0.015912  0.000000        0.00000
SCALE3      0.000000  0.000000  0.014151        0.00000
ATOM      1  N   SER     2      28.888   9.409  52.301  1.00 85.05
ATOM      2  CA  SER     2      27.638  10.125  52.516  1.00 80.05
ATOM      3  C   SER     2      26.499   9.639  51.644  1.00 85.36
ATOM      4  O   SER     2      26.606   8.656  50.915  1.00 84.56
ATOM      5  CB  SER     2      27.783  11.635  52.378  1.00 70.97
ATOM      6  OG  SER     2      27.690  12.033  51.012  1.00 44.08
ATOM      7  N   LYS     3      25.418  10.403  51.731  1.00 87.71
ATOM      8  CA  LYS     3      24.141  10.191  51.036  1.00 87.15
ATOM      9  C   LYS     3      24.214  10.266  49.497  1.00 76.86
ATOM     10  O   LYS     3      24.107   9.258  48.774  1.00 78.27
ATOM     11  CB  LYS     3      23.127  11.240  51.521  1.00 89.44
ATOM     12  CG  LYS     3      21.768  10.697  51.949  1.00 75.06
ATOM     13  CD  LYS     3      20.681  11.781  51.987  1.00 76.58
ATOM     14  CE  LYS     3      20.711  12.655  53.243  1.00 68.55
ATOM     15  NZ  LYS     3      20.816  14.103  52.953  1.00 46.24
ATOM     16  N   GLY     4      24.318  11.495  49.015  1.00 53.62
ATOM     17  CA  GLY     4      24.297  11.798  47.605  1.00 45.97
ATOM     18  C   GLY     4      25.425  11.206  46.796  1.00 31.90
ATOM     19  O   GLY     4      25.234  10.923  45.619  1.00 33.63
ATOM     20  N   GLU     5      26.606  11.082  47.420  1.00 32.54
ATOM     21  CA  GLU     5      27.821  10.598  46.726  1.00 32.57
ATOM     22  C   GLU     5      27.523   9.590  45.616  1.00 28.40
ATOM     23  O   GLU     5      27.850   9.803  44.444  1.00 26.12
ATOM     24  CB  GLU     5      28.873  10.053  47.718  1.00 38.53
ATOM     25  CG  GLU     5      30.337  10.461  47.425  1.00 41.36
ATOM     26  CD  GLU     5      31.311   9.584  48.170  1.00 90.82
ATOM     27  OE1 GLU     5      31.508   9.677  49.381  1.00 74.80
ATOM     28  OE2 GLU     5      31.839   8.653  47.403  1.00100.00
ATOM     29  N   GLU     6      26.883   8.499  46.017  1.00 28.57
ATOM     30  CA  GLU     6      26.479   7.410  45.150  1.00 31.50
ATOM     31  C   GLU     6      25.561   7.837  43.979  1.00 31.10
ATOM     32  O   GLU     6      25.479   7.142  42.955  1.00 30.96
ATOM     33  CB  GLU     6      25.780   6.330  45.992  1.00 35.64
ATOM     34  CG  GLU     6      25.260   6.893  47.338  1.00 55.53
ATOM     35  N   LEU     7      24.864   8.966  44.138  1.00 22.26
ATOM     36  CA  LEU     7      23.954   9.456  43.089  1.00 21.61
ATOM     37  C   LEU     7      24.693  10.061  41.917  1.00 16.90
ATOM     38  O   LEU     7      24.152  10.250  40.836  1.00 18.38
ATOM     39  CB  LEU     7      23.050  10.548  43.665  1.00 22.41
ATOM     40  CG  LEU     7      21.672  10.058  44.098  1.00 32.84
ATOM     41  CD1 LEU     7      21.597   8.536  44.074  1.00 31.64
ATOM     42  CD2 LEU     7      21.332  10.591  45.485  1.00 33.14
ATOM     43  N   PHE     8      25.944  10.407  42.157  1.00 20.75
ATOM     44  CA  PHE     8      26.740  11.132  41.159  1.00 21.64
ATOM     45  C   PHE     8      27.818  10.333  40.427  1.00 30.59
ATOM     46  O   PHE     8      28.590  10.856  39.600  1.00 30.05
ATOM     47  CB  PHE     8      27.309  12.376  41.820  1.00 16.95
ATOM     48  CG  PHE     8      26.222  13.355  42.163  1.00 13.29
ATOM     49  CD1 PHE     8      25.672  13.378  43.447  1.00 17.27
ATOM     50  CD2 PHE     8      25.726  14.227  41.189  1.00 13.12
ATOM     51  CE1 PHE     8      24.661  14.290  43.772  1.00 15.14
ATOM     52  CE2 PHE     8      24.712  15.137  41.499  1.00 13.19
ATOM     53  CZ  PHE     8      24.192  15.170  42.794  1.00  8.69
ATOM     54  N   THR     9      27.798   9.074  40.699  1.00 27.38
ATOM     55  CA  THR     9      28.704   8.122  40.175  1.00 34.93
ATOM     56  C   THR     9      28.709   7.998  38.636  1.00 45.22
ATOM     57  O   THR     9      29.642   7.452  38.062  1.00 50.55
ATOM     58  CB  THR     9      28.447   6.795  40.892  1.00 44.60
ATOM     59  OG1 THR     9      29.639   6.330  41.527  1.00 40.40
ATOM     60  CG2 THR     9      27.301   5.779  39.959  1.00 39.76
```

FIG 5-2

ATOM	61	N	GLY	10	27.690	8.510	37.956	1.00	30.53
ATOM	62	CA	GLY	10	27.689	8.458	36.507	1.00	23.21
ATOM	63	C	GLY	10	27.144	9.746	35.914	1.00	16.55
ATOM	64	O	GLY	10	27.011	10.729	36.617	1.00	25.70
ATOM	65	N	VAL	11	26.835	9.719	34.629	1.00	16.39
ATOM	66	CA	VAL	11	26.209	10.863	33.971	1.00	22.28
ATOM	67	C	VAL	11	24.758	11.020	34.479	1.00	29.60
ATOM	68	O	VAL	11	23.972	10.062	34.456	1.00	20.43
ATOM	69	CB	VAL	11	26.173	10.664	32.467	1.00	30.87
ATOM	70	CG1	VAL	11	25.912	11.980	31.734	1.00	31.75
ATOM	71	CG2	VAL	11	27.480	10.048	32.015	1.00	33.85
ATOM	72	N	VAL	12	24.417	12.227	34.931	1.00	20.12
ATOM	73	CA	VAL	12	23.080	12.561	35.433	1.00	12.88
ATOM	74	C	VAL	12	22.407	13.624	34.516	1.00	14.37
ATOM	75	O	VAL	12	23.007	14.639	34.179	1.00	13.42
ATOM	76	CB	VAL	12	23.270	13.077	36.839	1.00	15.01
ATOM	77	CG1	VAL	12	22.000	13.662	37.422	1.00	17.57
ATOM	78	CG2	VAL	12	23.781	11.936	37.728	1.00	16.55
ATOM	79	N	PRO	13	21.180	13.382	34.066	1.00	14.72
ATOM	80	CA	PRO	13	20.493	14.382	33.265	1.00	10.76
ATOM	81	C	PRO	13	20.116	15.589	34.141	1.00	7.65
ATOM	82	O	PRO	13	19.797	15.468	35.337	1.00	15.14
ATOM	83	CB	PRO	13	19.225	13.707	32.745	1.00	17.36
ATOM	84	CG	PRO	13	19.043	12.422	33.550	1.00	19.69
ATOM	85	CD	PRO	13	20.315	12.195	34.340	1.00	15.41
ATOM	86	N	ILE	14	20.196	16.766	33.557	1.00	14.91
ATOM	87	CA	ILE	14	19.893	17.991	34.266	1.00	12.93
ATOM	88	C	ILE	14	18.768	18.760	33.596	1.00	12.08
ATOM	89	O	ILE	14	18.724	18.878	32.399	1.00	11.04
ATOM	90	CB	ILE	14	21.109	18.905	34.325	1.00	16.54
ATOM	91	CG1	ILE	14	22.271	18.169	35.015	1.00	18.08
ATOM	92	CG2	ILE	14	20.783	20.207	35.084	1.00	11.56
ATOM	93	CD1	ILE	14	23.642	18.836	34.738	1.00	16.15
ATOM	94	N	LEU	15	17.899	19.307	34.421	1.00	13.85
ATOM	95	CA	LEU	15	16.811	20.136	33.955	1.00	14.82
ATOM	96	C	LEU	15	16.915	21.474	34.685	1.00	3.62
ATOM	97	O	LEU	15	17.080	21.509	35.901	1.00	10.00
ATOM	98	CB	LEU	15	15.462	19.450	34.285	1.00	21.25
ATOM	99	CG	LEU	15	14.412	19.541	33.199	1.00	40.50
ATOM	100	CD1	LEU	15	13.279	20.440	33.679	1.00	46.97
ATOM	101	CD2	LEU	15	15.008	20.098	31.913	1.00	49.22
ATOM	102	N	VAL	16	16.885	22.556	33.919	1.00	10.56
ATOM	103	CA	VAL	16	16.964	23.905	34.479	1.00	10.23
ATOM	104	C	VAL	16	15.716	24.727	34.063	1.00	9.47
ATOM	105	O	VAL	16	15.347	24.748	32.904	1.00	16.72
ATOM	106	CB	VAL	16	18.273	24.668	34.098	1.00	12.85
ATOM	107	CG1	VAL	16	18.226	26.075	34.691	1.00	12.58
ATOM	108	CG2	VAL	16	19.520	23.945	34.628	1.00	14.24
ATOM	109	N	GLU	17	15.059	25.317	35.060	1.00	14.43
ATOM	110	CA	GLU	17	13.904	26.144	34.870	1.00	13.61
ATOM	111	C	GLU	17	14.086	27.474	35.571	1.00	9.38
ATOM	112	O	GLU	17	14.331	27.524	36.765	1.00	15.74
ATOM	113	CB	GLU	17	12.650	25.402	35.344	1.00	14.15
ATOM	114	CG	GLU	17	12.436	24.178	34.447	1.00	15.37
ATOM	115	CD	GLU	17	11.865	24.573	33.105	1.00	49.50
ATOM	116	OE1	GLU	17	11.160	25.557	32.950	1.00	83.46
ATOM	117	OE2	GLU	17	12.220	23.766	32.127	1.00	38.75
ATOM	118	N	LEU	18	13.990	28.571	34.805	1.00	17.82
ATOM	119	CA	LEU	18	14.116	29.914	35.401	1.00	16.61
ATOM	120	C	LEU	18	12.962	30.855	35.057	1.00	14.91
ATOM	121	O	LEU	18	12.585	30.978	33.917	1.00	14.31
ATOM	122	CB	LEU	18	15.426	30.630	35.005	1.00	13.56
ATOM	123	CG	LEU	18	15.533	32.049	35.579	1.00	19.27
ATOM	124	CD1	LEU	18	16.740	32.182	36.489	1.00	21.40
ATOM	125	CD2	LEU	18	15.682	33.033	34.438	1.00	18.38
ATOM	126	N	ASP	19	12.480	31.551	36.082	1.00	17.88
ATOM	127	CA	ASP	19	11.476	32.577	35.940	1.00	19.57

FIG 5-3

ATOM	128	C	ASP	19	12.098	33.896	36.360	1.00	11.65
ATOM	129	O	ASP	19	12.486	34.044	37.493	1.00	16.82
ATOM	130	CB	ASP	19	10.234	32.305	36.847	1.00	24.92
ATOM	131	CG	ASP	19	9.305	31.262	36.282	1.00	38.46
ATOM	132	OD1	ASP	19	8.572	30.587	36.989	1.00	61.49
ATOM	133	OD2	ASP	19	9.337	31.189	34.949	1.00	22.44
ATOM	134	N	GLY	20	12.178	34.863	35.471	1.00	16.82
ATOM	135	CA	GLY	20	12.784	36.101	35.908	1.00	19.52
ATOM	136	C	GLY	20	12.048	37.385	35.538	1.00	19.35
ATOM	137	O	GLY	20	11.240	37.443	34.628	1.00	18.22
ATOM	138	N	ASP	21	12.401	38.407	36.286	1.00	13.19
ATOM	139	CA	ASP	21	11.908	39.737	36.112	1.00	16.36
ATOM	140	C	ASP	21	13.039	40.683	36.424	1.00	12.77
ATOM	141	O	ASP	21	13.517	40.742	37.569	1.00	15.18
ATOM	142	CB	ASP	21	10.701	40.036	37.040	1.00	22.26
ATOM	143	CG	ASP	21	10.230	41.491	37.022	1.00	30.80
ATOM	144	OD1	ASP	21	10.878	42.407	36.557	1.00	27.40
ATOM	145	OD2	ASP	21	9.062	41.658	37.604	1.00	45.92
ATOM	146	N	VAL	22	13.464	41.393	35.397	1.00	13.66
ATOM	147	CA	VAL	22	14.524	42.388	35.542	1.00	25.10
ATOM	148	C	VAL	22	14.010	43.780	35.154	1.00	18.26
ATOM	149	O	VAL	22	13.769	44.062	33.955	1.00	15.10
ATOM	150	CB	VAL	22	15.803	42.012	34.750	1.00	26.57
ATOM	151	CG1	VAL	22	16.861	43.127	34.896	1.00	24.27
ATOM	152	CG2	VAL	22	16.365	40.710	35.297	1.00	22.98
ATOM	153	N	ASN	23	13.823	44.641	36.166	1.00	25.32
ATOM	154	CA	ASN	23	13.319	45.993	35.908	1.00	32.81
ATOM	155	C	ASN	23	11.987	45.958	35.142	1.00	32.77
ATOM	156	O	ASN	23	11.774	46.730	34.187	1.00	30.47
ATOM	157	CB	ASN	23	14.344	46.831	35.096	1.00	31.26
ATOM	158	CG	ASN	23	15.374	47.607	35.938	1.00	24.72
ATOM	159	OD1	ASN	23	15.795	47.183	37.024	1.00	27.22
ATOM	160	ND2	ASN	23	15.829	48.723	35.389	1.00	41.15
ATOM	161	N	GLY	24	11.118	45.024	35.519	1.00	24.95
ATOM	162	CA	GLY	24	9.831	44.919	34.848	1.00	23.22
ATOM	163	C	GLY	24	9.832	44.111	33.573	1.00	23.31
ATOM	164	O	GLY	24	8.780	43.868	33.024	1.00	28.37
ATOM	165	N	HIS	25	11.000	43.691	33.071	1.00	20.89
ATOM	166	CA	HIS	25	11.042	42.840	31.877	1.00	19.30
ATOM	167	C	HIS	25	10.981	41.373	32.316	1.00	27.26
ATOM	168	O	HIS	25	11.898	40.850	32.951	1.00	26.47
ATOM	169	CB	HIS	25	12.268	43.060	30.958	1.00	24.20
ATOM	170	CG	HIS	25	12.313	44.382	30.218	1.00	33.04
ATOM	171	ND1	HIS	25	12.917	45.514	30.758	1.00	37.58
ATOM	172	CD2	HIS	25	11.876	44.716	28.971	1.00	42.76
ATOM	173	CE1	HIS	25	12.801	46.497	29.867	1.00	39.14
ATOM	174	NE2	HIS	25	12.185	46.050	28.778	1.00	42.80
ATOM	175	N	LYS	26	9.872	40.728	32.028	1.00	25.90
ATOM	176	CA	LYS	26	9.675	39.355	32.446	1.00	26.27
ATOM	177	C	LYS	26	10.154	38.361	31.429	1.00	27.09
ATOM	178	O	LYS	26	10.027	38.576	30.232	1.00	25.75
ATOM	179	CB	LYS	26	8.230	39.069	32.863	1.00	27.58
ATOM	180	CG	LYS	26	7.873	39.770	34.166	1.00	44.94
ATOM	181	CD	LYS	26	6.369	39.914	34.400	1.00	71.44
ATOM	182	CE	LYS	26	6.008	41.000	35.421	1.00	45.29
ATOM	183	N	PHE	27	10.703	37.250	31.910	1.00	22.04
ATOM	184	CA	PHE	27	11.164	36.236	30.978	1.00	18.78
ATOM	185	C	PHE	27	11.273	34.863	31.619	1.00	14.75
ATOM	186	O	PHE	27	11.293	34.722	32.842	1.00	15.94
ATOM	187	CB	PHE	27	12.495	36.638	30.287	1.00	21.58
ATOM	188	CG	PHE	27	13.599	36.826	31.311	1.00	22.06
ATOM	189	CD1	PHE	27	14.490	35.791	31.612	1.00	23.61
ATOM	190	CD2	PHE	27	13.722	38.029	32.005	1.00	17.85
ATOM	191	CE1	PHE	27	15.487	35.963	32.579	1.00	16.61
ATOM	192	CE2	PHE	27	14.747	38.234	32.931	1.00	18.75
ATOM	193	CZ	PHE	27	15.621	37.187	33.234	1.00	13.83
ATOM	194	N	SER	28	11.370	33.857	30.752	1.00	12.40

FIG 5-4

ATOM	195	CA	SER	28	11.492	32.479	31.186	1.00	15.59
ATOM	196	C	SER	28	12.579	31.749	30.379	1.00	15.96
ATOM	197	O	SER	28	12.699	31.933	29.167	1.00	18.99
ATOM	198	CB	SER	28	10.143	31.702	31.086	1.00	14.48
ATOM	199	OG	SER	28	9.510	31.678	32.353	1.00	31.95
ATOM	200	N	VAL	29	13.335	30.902	31.073	1.00	16.73
ATOM	201	CA	VAL	29	14.361	30.093	30.435	1.00	14.06
ATOM	202	C	VAL	29	14.258	28.614	30.817	1.00	6.80
ATOM	203	O	VAL	29	14.058	28.266	31.987	1.00	10.85
ATOM	204	CB	VAL	29	15.768	30.570	30.839	1.00	17.96
ATOM	205	CG1	VAL	29	16.826	29.599	30.234	1.00	15.30
ATOM	206	CG2	VAL	29	15.989	32.001	30.357	1.00	16.37
ATOM	207	N	SER	30	14.462	27.781	29.824	1.00	11.31
ATOM	208	CA	SER	30	14.535	26.351	30.011	1.00	17.96
ATOM	209	C	SER	30	15.917	25.818	29.571	1.00	11.26
ATOM	210	O	SER	30	16.398	26.157	28.513	1.00	13.17
ATOM	211	CB	SER	30	13.471	25.603	29.202	1.00	19.91
ATOM	212	OG	SER	30	12.249	25.667	29.882	1.00	48.74
ATOM	213	N	GLY	31	16.480	24.926	30.364	1.00	9.88
ATOM	214	CA	GLY	31	17.718	24.321	29.977	1.00	12.44
ATOM	215	C	GLY	31	17.737	22.816	30.249	1.00	13.16
ATOM	216	O	GLY	31	17.149	22.324	31.176	1.00	12.41
ATOM	217	N	GLU	32	18.459	22.112	29.433	1.00	13.44
ATOM	218	CA	GLU	32	18.622	20.670	29.570	1.00	13.73
ATOM	219	C	GLU	32	20.079	20.297	29.262	1.00	17.33
ATOM	220	O	GLU	32	20.734	20.946	28.456	1.00	15.56
ATOM	221	CB	GLU	32	17.761	19.893	28.543	1.00	12.67
ATOM	222	CG	GLU	32	16.264	20.187	28.618	1.00	26.43
ATOM	223	CD	GLU	32	15.501	19.547	27.468	1.00	21.13
ATOM	224	OE1	GLU	32	15.996	18.767	26.698	1.00	23.45
ATOM	225	OE2	GLU	32	14.292	20.022	27.337	1.00	30.63
ATOM	226	N	GLY	33	20.534	19.207	29.822	1.00	15.36
ATOM	227	CA	GLY	33	21.860	18.687	29.518	1.00	12.84
ATOM	228	C	GLY	33	22.236	17.602	30.467	1.00	14.69
ATOM	229	O	GLY	33	21.390	16.919	31.011	1.00	13.56
ATOM	230	N	GLU	34	23.525	17.453	30.702	1.00	15.15
ATOM	231	CA	GLU	34	23.971	16.450	31.621	1.00	18.14
ATOM	232	C	GLU	34	25.220	16.874	32.367	1.00	16.26
ATOM	233	O	GLU	34	25.926	17.760	31.944	1.00	18.67
ATOM	234	CB	GLU	34	24.180	15.114	30.927	1.00	22.53
ATOM	235	CG	GLU	34	24.948	15.261	29.624	1.00	33.78
ATOM	236	CD	GLU	34	24.879	14.020	28.796	1.00	55.15
ATOM	237	OE1	GLU	34	25.861	13.352	28.534	1.00	45.39
ATOM	238	OE2	GLU	34	23.653	13.719	28.430	1.00	56.26
ATOM	239	N	GLY	35	25.461	16.222	33.485	1.00	11.20
ATOM	240	CA	GLY	35	26.611	16.502	34.315	1.00	10.62
ATOM	241	C	GLY	35	27.293	15.192	34.662	1.00	19.92
ATOM	242	O	GLY	35	26.650	14.161	34.750	1.00	16.69
ATOM	243	N	ASP	36	28.594	15.238	34.860	1.00	16.92
ATOM	244	CA	ASP	36	29.367	14.061	35.221	1.00	16.19
ATOM	245	C	ASP	36	30.396	14.505	36.233	1.00	13.94
ATOM	246	O	ASP	36	31.469	15.004	35.879	1.00	15.77
ATOM	247	CB	ASP	36	30.032	13.457	33.948	1.00	19.98
ATOM	248	CG	ASP	36	30.681	12.066	34.075	1.00	31.92
ATOM	249	OD1	ASP	36	31.236	11.519	33.141	1.00	30.97
ATOM	250	OD2	ASP	36	30.587	11.515	35.248	1.00	25.32
ATOM	251	N	ALA	37	30.015	14.402	37.490	1.00	13.40
ATOM	252	CA	ALA	37	30.818	14.846	38.582	1.00	12.98
ATOM	253	C	ALA	37	32.181	14.145	38.637	1.00	21.94
ATOM	254	O	ALA	37	33.084	14.604	39.331	1.00	13.61
ATOM	255	CB	ALA	37	30.070	14.741	39.916	1.00	11.49
ATOM	256	N	THR	38	32.307	13.016	37.945	1.00	15.63
ATOM	257	CA	THR	38	33.581	12.290	37.943	1.00	19.94
ATOM	258	C	THR	38	34.705	13.114	37.335	1.00	25.61
ATOM	259	O	THR	38	35.850	13.069	37.775	1.00	17.99
ATOM	260	CB	THR	38	33.462	10.898	37.299	1.00	22.57
ATOM	261	OG1	THR	38	32.543	10.146	38.067	1.00	29.86

FIG 5-5

ATOM	262	CG2	THR	38	34.821	10.213	37.355	1.00	22.90
ATOM	263	N	TYR	39	34.323	13.920	36.347	1.00	18.45
ATOM	264	CA	TYR	39	35.210	14.837	35.675	1.00	9.39
ATOM	265	C	TYR	39	34.874	16.291	35.991	1.00	14.41
ATOM	266	O	TYR	39	35.454	17.177	35.410	1.00	16.24
ATOM	267	CB	TYR	39	35.156	14.582	34.180	1.00	11.82
ATOM	268	CG	TYR	39	35.426	13.137	33.929	1.00	28.73
ATOM	269	CD1	TYR	39	36.715	12.633	34.065	1.00	33.75
ATOM	270	CD2	TYR	39	34.392	12.249	33.642	1.00	39.19
ATOM	271	CE1	TYR	39	36.982	11.276	33.828	1.00	29.75
ATOM	272	CE2	TYR	39	34.635	10.885	33.435	1.00	45.41
ATOM	273	CZ	TYR	39	35.943	10.410	33.570	1.00	57.62
ATOM	274	OH	TYR	39	36.199	9.070	33.364	1.00	70.77
ATOM	275	N	GLY	40	33.935	16.525	36.929	1.00	9.94
ATOM	276	CA	GLY	40	33.474	17.879	37.266	1.00	7.02
ATOM	277	C	GLY	40	32.952	18.600	36.004	1.00	9.45
ATOM	278	O	GLY	40	33.068	19.830	35.829	1.00	12.63
ATOM	279	N	LYS	41	32.380	17.823	35.092	1.00	5.44
ATOM	280	CA	LYS	41	31.954	18.335	33.842	1.00	6.63
ATOM	281	C	LYS	41	30.414	18.554	33.703	1.00	20.92
ATOM	282	O	LYS	41	29.617	17.693	34.085	1.00	12.94
ATOM	283	CB	LYS	41	32.360	17.357	32.827	1.00	8.27
ATOM	284	CG	LYS	41	32.099	17.771	31.419	1.00	13.19
ATOM	285	CD	LYS	41	32.521	16.644	30.481	1.00	20.20
ATOM	286	CE	LYS	41	32.690	17.068	29.032	1.00	35.79
ATOM	287	NZ	LYS	41	33.113	15.954	28.147	1.00	47.56
ATOM	288	N	LEU	42	30.049	19.684	33.069	1.00	18.31
ATOM	289	CA	LEU	42	28.643	20.064	32.794	1.00	16.08
ATOM	290	C	LEU	42	28.456	20.422	31.330	1.00	14.23
ATOM	291	O	LEU	42	29.240	21.168	30.787	1.00	14.79
ATOM	292	CB	LEU	42	28.223	21.300	33.621	1.00	13.22
ATOM	293	CG	LEU	42	28.007	21.061	35.082	1.00	16.70
ATOM	294	CD1	LEU	42	27.894	22.406	35.782	1.00	13.79
ATOM	295	CD2	LEU	42	26.732	20.243	35.295	1.00	18.70
ATOM	296	N	THR	43	27.395	19.914	30.672	1.00	8.04
ATOM	297	CA	THR	43	27.103	20.275	29.282	1.00	4.87
ATOM	298	C	THR	43	25.636	20.666	29.186	1.00	17.23
ATOM	299	O	THR	43	24.811	19.818	29.442	1.00	14.36
ATOM	300	CB	THR	43	27.351	19.140	28.317	1.00	21.59
ATOM	301	OG1	THR	43	28.692	18.743	28.415	1.00	42.74
ATOM	302	CG2	THR	43	27.073	19.675	26.917	1.00	31.23
ATOM	303	N	LEU	44	25.327	21.934	28.830	1.00	11.83
ATOM	304	CA	LEU	44	23.944	22.409	28.847	1.00	13.81
ATOM	305	C	LEU	44	23.589	23.307	27.668	1.00	18.19
ATOM	306	O	LEU	44	24.416	23.989	27.107	1.00	13.86
ATOM	307	CB	LEU	44	23.725	23.275	30.125	1.00	15.37
ATOM	308	CG	LEU	44	23.369	22.584	31.456	1.00	24.69
ATOM	309	CD1	LEU	44	21.869	22.381	31.601	1.00	23.20
ATOM	310	CD2	LEU	44	24.083	21.286	31.650	1.00	46.18
ATOM	311	N	LYS	45	22.294	23.331	27.339	1.00	10.29
ATOM	312	CA	LYS	45	21.752	24.224	26.358	1.00	11.94
ATOM	313	C	LYS	45	20.534	24.913	26.957	1.00	19.35
ATOM	314	O	LYS	45	19.665	24.248	27.530	1.00	18.43
ATOM	315	CB	LYS	45	21.409	23.560	25.060	1.00	13.75
ATOM	316	CG	LYS	45	20.878	24.556	24.045	1.00	8.83
ATOM	317	CD	LYS	45	20.486	23.863	22.746	1.00	26.87
ATOM	318	CE	LYS	45	19.574	24.688	21.842	1.00	16.58
ATOM	319	NZ	LYS	45	19.318	24.024	20.555	1.00	18.33
ATOM	320	N	PHE	46	20.535	26.236	26.910	1.00	12.34
ATOM	321	CA	PHE	46	19.463	27.048	27.451	1.00	13.32
ATOM	322	C	PHE	46	18.759	27.718	26.343	1.00	18.26
ATOM	323	O	PHE	46	19.326	28.093	25.360	1.00	16.83
ATOM	324	CB	PHE	46	19.934	28.101	28.473	1.00	15.29
ATOM	325	CG	PHE	46	20.773	27.495	29.552	1.00	13.91
ATOM	326	CD1	PHE	46	22.132	27.268	29.337	1.00	17.06
ATOM	327	CD2	PHE	46	20.209	27.121	30.774	1.00	8.24
ATOM	328	CE1	PHE	46	22.924	26.693	30.331	1.00	15.95

FIG 5-6

```
ATOM    329  CE2 PHE    46      20.979  26.524  31.767  1.00 11.90
ATOM    330  CZ  PHE    46      22.340  26.309  31.540  1.00  8.84
ATOM    331  N   ILE    47      17.440  27.845  26.498  1.00 13.24
ATOM    332  CA  ILE    47      16.588  28.453  25.479  1.00 18.02
ATOM    333  C   ILE    47      15.645  29.460  26.118  1.00 20.14
ATOM    334  O   ILE    47      15.039  29.162  27.148  1.00 17.67
ATOM    335  CB  ILE    47      15.737  27.386  24.801  1.00 22.67
ATOM    336  CG1 ILE    47      16.585  26.271  24.291  1.00 20.66
ATOM    337  CG2 ILE    47      15.024  28.002  23.641  1.00 33.79
ATOM    338  CD1 ILE    47      16.639  26.293  22.805  1.00 23.69
ATOM    339  N   CYS    48      15.564  30.653  25.561  1.00 14.68
ATOM    340  CA  CYS    48      14.681  31.635  26.170  1.00 16.93
ATOM    341  C   CYS    48      13.323  31.352  25.628  1.00 24.18
ATOM    342  O   CYS    48      13.122  31.513  24.453  1.00 20.63
ATOM    343  CB  CYS    48      15.063  33.116  25.885  1.00 16.85
ATOM    344  SG  CYS    48      13.913  34.268  26.712  1.00 22.06
ATOM    345  N   THR    49      12.424  30.871  26.484  1.00 27.31
ATOM    346  CA  THR    49      11.101  30.458  26.042  1.00 32.18
ATOM    347  C   THR    49      10.106  31.572  25.803  1.00 37.51
ATOM    348  O   THR    49       9.150  31.407  25.061  1.00 35.71
ATOM    349  CB  THR    49      10.537  29.417  26.972  1.00 23.66
ATOM    350  OG1 THR    49      10.387  29.989  28.258  1.00 30.10
ATOM    351  CG2 THR    49      11.512  28.226  27.022  1.00 29.98
ATOM    352  N   THR    50      10.314  32.693  26.447  1.00 32.34
ATOM    353  CA  THR    50       9.416  33.810  26.283  1.00 28.67
ATOM    354  C   THR    50       9.836  34.711  25.126  1.00 37.98
ATOM    355  O   THR    50       9.228  35.763  24.904  1.00 39.17
ATOM    356  CB  THR    50       9.251  34.611  27.589  1.00 36.23
ATOM    357  OG1 THR    50      10.512  34.980  28.118  1.00 35.37
ATOM    358  CG2 THR    50       8.507  33.773  28.602  1.00 27.78
ATOM    359  N   GLY    51      10.881  34.282  24.372  1.00 31.04
ATOM    360  CA  GLY    51      11.394  35.059  23.239  1.00 32.42
ATOM    361  C   GLY    51      12.865  35.542  23.427  1.00 48.45
ATOM    362  O   GLY    51      13.779  34.737  23.701  1.00 57.11
ATOM    363  N   LYS    52      13.087  36.862  23.282  1.00 36.08
ATOM    364  CA  LYS    52      14.416  37.460  23.416  1.00 35.75
ATOM    365  C   LYS    52      14.827  37.726  24.861  1.00 29.65
ATOM    366  O   LYS    52      14.140  38.420  25.620  1.00 25.70
ATOM    367  CB  LYS    52      14.577  38.714  22.582  1.00 43.37
ATOM    368  CG  LYS    52      15.772  38.649  21.644  1.00 78.17
ATOM    369  N   LEU    53      15.983  37.190  25.250  1.00 19.22
ATOM    370  CA  LEU    53      16.439  37.430  26.596  1.00 13.52
ATOM    371  C   LEU    53      16.717  38.932  26.775  1.00 17.76
ATOM    372  O   LEU    53      17.392  39.539  25.973  1.00 21.59
ATOM    373  CB  LEU    53      17.705  36.567  26.845  1.00 17.39
ATOM    374  CG  LEU    53      18.100  36.435  28.302  1.00 17.43
ATOM    375  CD1 LEU    53      17.048  35.621  29.053  1.00 20.12
ATOM    376  CD2 LEU    53      19.440  35.718  28.368  1.00 16.11
ATOM    377  N   PRO    54      16.197  39.525  27.817  1.00 16.69
ATOM    378  CA  PRO    54      16.324  40.962  28.092  1.00 18.60
ATOM    379  C   PRO    54      17.638  41.414  28.707  1.00 25.39
ATOM    380  O   PRO    54      17.865  42.609  28.861  1.00 18.88
ATOM    381  CB  PRO    54      15.268  41.265  29.139  1.00 22.52
ATOM    382  CG  PRO    54      14.832  39.933  29.720  1.00 26.02
ATOM    383  CD  PRO    54      15.318  38.855  28.779  1.00 21.26
ATOM    384  N   VAL    55      18.435  40.455  29.161  1.00 23.32
ATOM    385  CA  VAL    55      19.746  40.716  29.711  1.00 15.83
ATOM    386  C   VAL    55      20.688  39.868  28.973  1.00 19.38
ATOM    387  O   VAL    55      20.268  39.035  28.219  1.00 20.34
ATOM    388  CB  VAL    55      19.814  40.409  31.147  1.00 17.67
ATOM    389  CG1 VAL    55      18.864  41.340  31.851  1.00 22.52
ATOM    390  CG2 VAL    55      19.402  38.959  31.397  1.00 19.11
ATOM    391  N   PRO    56      21.963  40.070  29.167  1.00 19.37
ATOM    392  CA  PRO    56      22.911  39.258  28.447  1.00 13.09
ATOM    393  C   PRO    56      23.059  37.834  29.038  1.00  5.83
ATOM    394  O   PRO    56      23.067  37.631  30.254  1.00 12.35
ATOM    395  CB  PRO    56      24.231  40.062  28.420  1.00 13.34
```

FIG 5-7

ATOM	396	CG	PRO	56	23.851	41.478	28.849	1.00	20.73
ATOM	397	CD	PRO	56	22.525	41.379	29.578	1.00	18.66
ATOM	398	N	TRP	57	23.202	36.848	28.158	1.00	11.12
ATOM	399	CA	TRP	57	23.354	35.458	28.595	1.00	12.38
ATOM	400	C	TRP	57	24.411	35.239	29.700	1.00	14.13
ATOM	401	O	TRP	57	24.178	34.586	30.709	1.00	11.49
ATOM	402	CB	TRP	57	23.604	34.535	27.406	1.00	10.56
ATOM	403	CG	TRP	57	22.335	34.237	26.641	1.00	12.68
ATOM	404	CD1	TRP	57	21.999	34.714	25.426	1.00	16.24
ATOM	405	CD2	TRP	57	21.281	33.327	27.013	1.00	12.50
ATOM	406	NE1	TRP	57	20.784	34.200	25.018	1.00	14.28
ATOM	407	CE2	TRP	57	20.315	33.354	25.963	1.00	14.68
ATOM	408	CE3	TRP	57	21.052	32.521	28.129	1.00	12.01
ATOM	409	CZ2	TRP	57	19.148	32.583	26.007	1.00	14.36
ATOM	410	CZ3	TRP	57	19.887	31.767	28.170	1.00	14.23
ATOM	411	CH2	TRP	57	18.945	31.818	27.128	1.00	10.01
ATOM	412	N	PRO	58	25.594	35.800	29.518	1.00	15.78
ATOM	413	CA	PRO	58	26.629	35.616	30.503	1.00	9.53
ATOM	414	C	PRO	58	26.241	36.010	31.878	1.00	9.71
ATOM	415	O	PRO	58	26.760	35.467	32.825	1.00	11.70
ATOM	416	CB	PRO	58	27.833	36.441	30.040	1.00	10.83
ATOM	417	CG	PRO	58	27.597	36.748	28.582	1.00	18.50
ATOM	418	CD	PRO	58	26.137	36.432	28.278	1.00	15.82
ATOM	419	N	THR	59	25.336	36.977	32.021	1.00	7.54
ATOM	420	CA	THR	59	24.976	37.366	33.357	1.00	4.53
ATOM	421	C	THR	59	24.228	36.258	34.137	1.00	8.41
ATOM	422	O	THR	59	24.174	36.261	35.367	1.00	10.57
ATOM	423	CB	THR	59	24.187	38.691	33.384	1.00	16.64
ATOM	424	OG1	THR	59	22.895	38.480	32.844	1.00	15.51
ATOM	425	CG2	THR	59	24.917	39.731	32.542	1.00	15.76
ATOM	426	N	LEU	60	23.686	35.304	33.427	1.00	11.99
ATOM	427	CA	LEU	60	22.899	34.248	34.073	1.00	9.15
ATOM	428	C	LEU	60	23.657	32.944	34.385	1.00	15.62
ATOM	429	O	LEU	60	23.118	32.027	35.042	1.00	11.99
ATOM	430	CB	LEU	60	21.645	33.914	33.203	1.00	7.67
ATOM	431	CG	LEU	60	20.728	35.111	33.042	1.00	14.06
ATOM	432	CD1	LEU	60	19.620	34.775	32.062	1.00	14.54
ATOM	433	CD2	LEU	60	20.142	35.456	34.394	1.00	10.67
ATOM	434	N	VAL	61	24.893	32.837	33.917	1.00	11.27
ATOM	435	CA	VAL	61	25.656	31.587	34.094	1.00	4.37
ATOM	436	C	VAL	61	25.678	31.013	35.496	1.00	6.02
ATOM	437	O	VAL	61	25.355	29.805	35.743	1.00	10.75
ATOM	438	CB	VAL	61	27.050	31.643	33.406	1.00	7.14
ATOM	439	CG1	VAL	61	27.888	30.396	33.805	1.00	6.47
ATOM	440	CG2	VAL	61	26.890	31.745	31.876	1.00	6.63
ATOM	441	N	THR	62	26.053	31.843	36.442	1.00	7.02
ATOM	442	CA	THR	62	26.178	31.421	37.808	1.00	6.51
ATOM	443	C	THR	62	24.862	30.954	38.410	1.00	9.22
ATOM	444	O	THR	62	24.801	30.163	39.352	1.00	6.99
ATOM	445	CB	THR	62	26.816	32.520	38.660	1.00	16.97
ATOM	446	OG1	THR	62	26.103	33.744	38.453	1.00	12.00
ATOM	447	CG2	THR	62	28.297	32.708	38.225	1.00	8.86
ATOM	448	N	THR	63	23.814	31.547	37.910	1.00	9.98
ATOM	449	CA	THR	63	22.457	31.212	38.388	1.00	6.69
ATOM	450	C	THR	63	22.033	29.830	37.865	1.00	8.14
ATOM	451	O	THR	63	21.499	28.984	38.604	1.00	13.48
ATOM	452	CB	THR	63	21.458	32.312	37.925	1.00	11.14
ATOM	453	OG1	THR	63	21.785	33.498	38.602	1.00	11.78
ATOM	454	CG2	THR	63	20.024	31.897	38.296	1.00	9.31
ATOM	455	N	PHE	64	22.250	29.620	36.583	1.00	10.19
ATOM	456	CA	PHE	64	21.895	28.371	35.995	1.00	8.00
ATOM	457	C	PHE	64	22.774	27.253	36.518	1.00	25.26
ATOM	458	O	PHE	64	22.313	26.147	36.761	1.00	9.64
ATOM	459	CB	PHE	64	22.114	28.438	34.513	1.00	6.88
ATOM	460	CG	PHE	64	21.233	29.357	33.750	1.00	10.96
ATOM	461	CD1	PHE	64	21.724	30.954	32.593	1.00	9.15
ATOM	462	CD2	PHE	64	19.899	29.563	34.106	1.00	14.43

FIG 5-8

ATOM	463	CE1	PHE	64	20.936	30.792	31.805	1.00	14.20
ATOM	464	CE2	PHE	64	19.077	30.375	33.317	1.00	13.95
ATOM	465	CZ	PHE	64	19.597	30.983	32.171	1.00	16.35
HETATM	466	N1	CRO	66	24.077	27.513	36.610	1.00	11.86
HETATM	467	CG1	CRO	66	25.155	25.422	34.796	1.00	16.67
HETATM	468	OG1	CRO	66	26.679	27.129	35.461	1.00	14.22
HETATM	469	CB1	CRO	66	25.931	26.035	35.930	1.00	10.77
HETATM	470	CA1	CRO	66	25.011	26.478	37.078	1.00	7.34
HETATM	471	C1	CRO	66	25.718	26.991	38.253	1.00	17.70
HETATM	472	N2	CRO	66	26.975	27.732	38.216	1.00	9.21
HETATM	473	OH	CRO	66	32.894	30.804	36.971	1.00	13.84
HETATM	474	CD2	CRO	66	30.487	30.110	39.805	1.00	10.79
HETATM	475	CE2	CRO	66	31.614	30.563	39.085	1.00	10.01
HETATM	476	CZ	CRO	66	31.718	30.300	37.721	1.00	9.48
HETATM	477	CE1	CRO	66	30.707	29.546	37.033	1.00	17.44
HETATM	478	CD1	CRO	66	29.541	29.103	37.742	1.00	11.31
HETATM	479	CG2	CRO	66	29.437	29.370	39.124	1.00	7.67
HETATM	480	CB2	CRO	66	28.329	28.822	39.960	1.00	10.75
HETATM	481	CA2	CRO	66	27.197	28.245	39.512	1.00	16.08
HETATM	482	C2	CRO	66	26.043	27.875	40.370	1.00	5.46
HETATM	483	O2	CRO	66	26.022	27.962	41.566	1.00	13.20
HETATM	484	N3	CRO	66	25.240	26.978	39.517	1.00	18.43
HETATM	485	CA3	CRO	66	23.840	26.511	39.734	1.00	10.40
HETATM	486	C3	CRO	66	23.413	25.550	40.817	1.00	11.96
HETATM	487	O3	CRO	66	22.747	26.014	41.764	1.00	100.00
ATOM	488	N	VAL	68	23.737	24.208	41.005	1.00	29.95
ATOM	489	CA	VAL	68	24.209	22.972	40.304	1.00	17.16
ATOM	490	C	VAL	68	25.692	22.550	40.734	1.00	14.88
ATOM	491	O	VAL	68	26.379	21.821	40.026	1.00	9.03
ATOM	492	CB	VAL	68	23.870	22.899	38.831	1.00	18.94
ATOM	493	CG1	VAL	68	24.685	22.088	37.942	1.00	17.17
ATOM	494	CG2	VAL	68	22.396	22.538	38.680	1.00	18.80
ATOM	495	N	GLN	69	26.129	22.965	41.914	1.00	11.04
ATOM	496	CA	GLN	69	27.465	22.764	42.394	1.00	15.00
ATOM	497	C	GLN	69	27.749	21.366	42.893	1.00	22.46
ATOM	498	O	GLN	69	28.876	21.026	43.154	1.00	15.84
ATOM	499	CB	GLN	69	27.929	23.852	43.414	1.00	10.93
ATOM	500	CG	GLN	69	28.202	25.174	42.615	1.00	14.13
ATOM	501	CD	GLN	69	28.216	26.385	43.520	1.00	17.01
ATOM	502	OE1	GLN	69	27.433	26.476	44.448	1.00	18.94
ATOM	503	NE2	GLN	69	29.151	27.300	43.241	1.00	8.52
ATOM	504	N	CYS	70	26.703	20.540	42.906	1.00	12.10
ATOM	505	CA	CYS	70	26.862	19.171	43.287	1.00	11.84
ATOM	506	C	CYS	70	27.611	18.391	42.175	1.00	10.54
ATOM	507	O	CYS	70	28.036	17.242	42.367	1.00	14.70
ATOM	508	CB	CYS	70	25.476	18.584	43.596	1.00	14.52
ATOM	509	SG	CYS	70	24.325	19.012	42.251	1.00	15.61
ATOM	510	N	PHE	71	27.801	19.029	41.005	1.00	8.64
ATOM	511	CA	PHE	71	28.525	18.419	39.883	1.00	6.59
ATOM	512	C	PHE	71	30.041	18.754	39.876	1.00	16.43
ATOM	513	O	PHE	71	30.753	18.481	38.916	1.00	13.05
ATOM	514	CB	PHE	71	27.951	18.771	38.523	1.00	7.61
ATOM	515	CG	PHE	71	26.669	18.016	38.303	1.00	14.73
ATOM	516	CD1	PHE	71	26.693	16.642	38.050	1.00	10.34
ATOM	517	CD2	PHE	71	25.434	18.660	38.453	1.00	17.14
ATOM	518	CE1	PHE	71	25.506	16.931	37.866	1.00	15.09
ATOM	519	CE2	PHE	71	24.238	17.961	38.300	1.00	20.92
ATOM	520	CZ	PHE	71	24.282	16.598	37.990	1.00	18.49
ATOM	521	N	SER	72	30.500	19.370	40.938	1.00	13.13
ATOM	522	CA	SER	72	31.889	19.715	41.075	1.00	11.65
ATOM	523	C	SER	72	32.689	18.446	41.357	1.00	14.56
ATOM	524	O	SER	72	32.256	17.566	42.122	1.00	10.90
ATOM	525	CB	SER	72	32.075	20.672	42.257	1.00	8.65
ATOM	526	OG	SER	72	31.361	21.874	42.038	1.00	19.29
ATOM	527	N	ARG	73	33.905	18.358	40.794	1.00	16.27
ATOM	528	CA	ARG	73	34.695	17.212	41.117	1.00	13.56
ATOM	529	C	ARG	73	35.414	17.426	42.443	1.00	19.96

FIG 5-9

ATOM	530	O	ARG	73	36.182	18.376	42.599	1.00	16.14
ATOM	531	CB	ARG	73	35.694	16.817	40.013	1.00	16.80
ATOM	532	CG	ARG	73	36.549	15.616	40.460	1.00	20.13
ATOM	533	CD	ARG	73	37.489	15.093	39.381	1.00	28.47
ATOM	534	NE	ARG	73	38.743	15.859	39.260	1.00	25.48
ATOM	535	CZ	ARG	73	39.756	15.777	40.127	1.00	28.04
ATOM	536	NH1	ARG	73	39.688	15.004	41.195	1.00	28.76
ATOM	537	NH2	ARG	73	40.865	16.504	39.918	1.00	39.65
ATOM	538	N	TYR	74	35.151	16.561	43.424	1.00	12.05
ATOM	539	CA	TYR	74	35.861	16.659	44.690	1.00	11.57
ATOM	540	C	TYR	74	36.946	15.566	44.721	1.00	25.02
ATOM	541	O	TYR	74	36.658	14.387	44.558	1.00	19.71
ATOM	542	CB	TYR	74	34.978	16.528	45.934	1.00	15.51
ATOM	543	CG	TYR	74	34.395	17.850	46.402	1.00	16.59
ATOM	544	CD1	TYR	74	33.455	18.546	45.631	1.00	14.44
ATOM	545	CD2	TYR	74	34.799	18.399	47.618	1.00	15.94
ATOM	546	CE1	TYR	74	32.901	19.756	46.059	1.00	7.99
ATOM	547	CE2	TYR	74	34.261	19.612	48.058	1.00	18.29
ATOM	548	CZ	TYR	74	33.294	20.276	47.298	1.00	13.87
ATOM	549	OH	TYR	74	32.829	21.507	47.738	1.00	18.39
ATOM	550	N	PRO	75	38.181	15.947	44.902	1.00	19.20
ATOM	551	CA	PRO	75	39.213	14.940	44.995	1.00	18.42
ATOM	552	C	PRO	75	38.958	13.993	46.175	1.00	15.60
ATOM	553	O	PRO	75	38.373	14.361	47.174	1.00	11.99
ATOM	554	CB	PRO	75	40.514	15.681	45.195	1.00	18.31
ATOM	555	CG	PRO	75	40.242	17.158	44.863	1.00	24.81
ATOM	556	CD	PRO	75	38.742	17.306	44.694	1.00	15.41
ATOM	557	N	ASP	76	39.433	12.756	46.038	1.00	18.63
ATOM	558	CA	ASP	76	39.269	11.770	47.062	1.00	16.19
ATOM	559	C	ASP	76	39.581	12.280	48.431	1.00	15.92
ATOM	560	O	ASP	76	38.862	12.042	49.389	1.00	17.35
ATOM	561	CB	ASP	76	40.083	10.507	46.790	1.00	18.69
ATOM	562	CG	ASP	76	39.826	9.432	47.825	1.00	24.04
ATOM	563	OD1	ASP	76	40.523	9.268	48.817	1.00	29.72
ATOM	564	OD2	ASP	76	38.732	8.743	47.584	1.00	40.96
ATOM	565	N	HIS	77	40.647	12.984	48.561	1.00	18.79
ATOM	566	CA	HIS	77	40.978	13.418	49.877	1.00	19.36
ATOM	567	C	HIS	77	40.117	14.507	50.397	1.00	24.57
ATOM	568	O	HIS	77	40.205	14.826	51.551	1.00	27.15
ATOM	569	CB	HIS	77	42.435	13.806	50.042	1.00	19.84
ATOM	570	CG	HIS	77	42.743	15.035	49.322	1.00	17.31
ATOM	571	ND1	HIS	77	42.925	15.028	47.953	1.00	21.86
ATOM	572	CD2	HIS	77	42.925	16.295	49.774	1.00	18.70
ATOM	573	CE1	HIS	77	43.203	16.289	47.593	1.00	17.49
ATOM	574	NE2	HIS	77	43.213	17.069	48.668	1.00	18.11
ATOM	575	N	MSE	78	39.277	15.069	49.565	1.00	25.36
ATOM	576	CA	MSE	78	38.412	16.140	50.026	1.00	24.65
ATOM	577	C	MSE	78	36.920	15.774	50.066	1.00	26.47
ATOM	578	O	MSE	78	36.070	16.636	50.260	1.00	28.16
ATOM	579	CB	MSE	78	38.596	17.331	49.121	1.00	26.38
ATOM	580	CG	MSE	78	39.803	18.177	49.406	1.00	27.01
ATOM	581	SE	MSE	78	39.987	19.608	48.117	1.00	43.09
ATOM	582	CE	MSE	78	38.874	20.873	49.044	1.00	27.11
ATOM	583	N	LYS	79	36.606	14.509	49.856	1.00	18.68
ATOM	584	CA	LYS	79	35.216	14.061	49.853	1.00	21.54
ATOM	585	C	LYS	79	34.406	14.449	51.082	1.00	20.21
ATOM	586	O	LYS	79	33.186	14.652	51.025	1.00	21.08
ATOM	587	CB	LYS	79	35.152	12.581	49.612	1.00	23.48
ATOM	588	CG	LYS	79	35.859	12.225	48.317	1.00	41.09
ATOM	589	CD	LYS	79	35.159	11.134	47.535	1.00	34.66
ATOM	590	CE	LYS	79	35.796	10.881	46.181	1.00	53.46
ATOM	591	NZ	LYS	79	35.084	11.549	45.080	1.00	49.53
ATOM	592	N	ARG	80	35.069	14.542	52.213	1.00	19.77
ATOM	593	CA	ARG	80	34.365	14.874	53.434	1.00	20.13
ATOM	594	C	ARG	80	33.898	16.311	53.481	1.00	26.42
ATOM	595	O	ARG	80	33.251	16.717	54.467	1.00	22.51
ATOM	596	CB	ARG	80	35.155	14.549	54.700	1.00	24.58

FIG 5-10

```
ATOM    597  CG  ARG    80      36.204  15.620  55.034  1.00 29.71
ATOM    598  CD  ARG    80      36.964  15.344  56.335  1.00 61.30
ATOM    599  NE  ARG    80      36.551  16.230  57.415  1.00 71.14
ATOM    600  CZ  ARG    80      37.398  16.882  58.192  1.00100.00
ATOM    601  NH1 ARG    80      38.714  16.758  58.040  1.00100.00
ATOM    602  NH2 ARG    80      36.917  17.679  59.155  1.00 99.06
ATOM    603  N   HIS    81      34.275  17.121  52.473  1.00 18.77
ATOM    604  CA  HIS    81      33.903  18.547  52.499  1.00 19.60
ATOM    605  C   HIS    81      32.841  18.883  51.486  1.00 18.62
ATOM    606  O   HIS    81      32.557  20.043  51.295  1.00 17.76
ATOM    607  CB  HIS    81      35.129  19.472  52.283  1.00 20.39
ATOM    608  CG  HIS    81      36.221  19.224  53.305  1.00 28.02
ATOM    609  ND1 HIS    81      36.127  19.701  54.618  1.00 30.59
ATOM    610  CD2 HIS    81      37.392  18.535  53.202  1.00 29.02
ATOM    611  CE1 HIS    81      37.218  19.308  55.265  1.00 26.24
ATOM    612  NE2 HIS    81      37.991  18.603  54.452  1.00 28.18
ATOM    613  N   ASP    82      32.298  17.843  50.841  1.00 12.20
ATOM    614  CA  ASP    82      31.358  18.011  49.769  1.00 13.24
ATOM    615  C   ASP    82      29.922  18.148  50.259  1.00 24.30
ATOM    616  O   ASP    82      29.175  17.195  50.243  1.00 16.55
ATOM    617  CB  ASP    82      31.480  16.917  48.730  1.00 12.23
ATOM    618  CG  ASP    82      30.642  17.209  47.518  1.00  9.92
ATOM    619  OD1 ASP    82      29.870  18.134  47.459  1.00 20.31
ATOM    620  OD2 ASP    82      30.938  16.466  46.507  1.00 11.12
ATOM    621  N   PHE    83      29.566  19.353  50.705  1.00 23.66
ATOM    622  CA  PHE    83      28.220  19.634  51.201  1.00 20.23
ATOM    623  C   PHE    83      27.154  19.333  50.168  1.00 20.93
ATOM    624  O   PHE    83      26.116  18.733  50.503  1.00 15.97
ATOM    625  CB  PHE    83      28.077  21.106  51.666  1.00 19.59
ATOM    626  CG  PHE    83      26.624  21.613  51.805  1.00 16.91
ATOM    627  CD1 PHE    83      25.946  21.498  53.021  1.00 17.76
ATOM    628  CD2 PHE    83      25.968  22.236  50.734  1.00 18.88
ATOM    629  CE1 PHE    83      24.635  21.960  53.156  1.00 24.13
ATOM    630  CE2 PHE    83      24.650  22.690  50.840  1.00 19.24
ATOM    631  CZ  PHE    83      24.001  22.575  52.068  1.00 20.67
ATOM    632  N   PHE    84      27.432  19.784  48.921  1.00 14.06
ATOM    633  CA  PHE    84      26.515  19.693  47.809  1.00 12.96
ATOM    634  C   PHE    84      25.893  18.332  47.602  1.00 24.96
ATOM    635  O   PHE    84      24.674  18.200  47.534  1.00 21.55
ATOM    636  CB  PHE    84      27.085  20.265  46.513  1.00 13.44
ATOM    637  CG  PHE    84      27.630  21.645  46.721  1.00 14.27
ATOM    638  CD1 PHE    84      29.001  21.845  46.890  1.00 15.17
ATOM    639  CD2 PHE    84      26.781  22.753  46.752  1.00 13.48
ATOM    640  CE1 PHE    84      29.520  23.129  47.073  1.00 14.63
ATOM    641  CE2 PHE    84      27.276  24.041  46.969  1.00 16.34
ATOM    642  CZ  PHE    84      28.650  24.221  47.137  1.00 15.77
ATOM    643  N   LYS    85      26.738  17.330  47.482  1.00 14.07
ATOM    644  CA  LYS    85      26.294  15.985  47.283  1.00 13.30
ATOM    645  C   LYS    85      25.657  15.371  48.547  1.00 13.43
ATOM    646  O   LYS    85      24.773  14.509  48.429  1.00 18.46
ATOM    647  CB  LYS    85      27.434  15.089  46.757  1.00 17.38
ATOM    648  CG  LYS    85      27.873  15.372  45.323  1.00 13.93
ATOM    649  CD  LYS    85      28.969  14.381  44.888  1.00 13.23
ATOM    650  CE  LYS    85      29.766  14.819  43.662  1.00 10.36
ATOM    651  NZ  LYS    85      30.319  16.185  43.773  1.00 12.92
ATOM    652  N   SER    86      26.119  15.795  49.752  1.00 11.03
ATOM    653  CA  SER    86      25.610  15.267  50.998  1.00 12.09
ATOM    654  C   SER    86      24.156  15.639  51.240  1.00 21.58
ATOM    655  O   SER    86      23.452  14.979  52.013  1.00 19.89
ATOM    656  CB  SER    86      26.448  15.661  52.208  1.00 16.45
ATOM    657  OG  SER    86      26.308  17.042  52.495  1.00 22.05
ATOM    658  N   ALA    87      23.705  16.698  50.582  1.00 15.09
ATOM    659  CA  ALA    87      22.333  17.138  50.762  1.00 19.52
ATOM    660  C   ALA    87      21.337  16.399  49.870  1.00 18.60
ATOM    661  O   ALA    87      20.162  16.557  50.040  1.00 19.55
ATOM    662  CB  ALA    87      22.204  18.647  50.632  1.00 19.22
ATOM    663  N   MSE    88      21.835  15.536  48.976  1.00 14.05
```

FIG 5-11

```
ATOM    664  CA  MSE    88      21.007  14.796  48.035  1.00 15.32
ATOM    665  C   MSE    88      20.496  13.448  48.579  1.00 21.48
ATOM    666  O   MSE    88      21.109  12.876  49.457  1.00 23.03
ATOM    667  CB  MSE    88      21.848  14.593  46.791  1.00 16.98
ATOM    668  CG  MSE    88      22.263  15.891  46.131  1.00 10.66
ATOM    669  SE  MSE    88      20.737  16.894  45.394  1.00 31.99
ATOM    670  CE  MSE    88      21.318  18.684  45.748  1.00 28.86
ATOM    671  N   PRO    89      19.363  12.930  48.084  1.00 14.78
ATOM    672  CA  PRO    89      18.552  13.475  47.008  1.00 14.80
ATOM    673  C   PRO    89      17.572  14.611  47.385  1.00 12.10
ATOM    674  O   PRO    89      17.085  15.301  46.493  1.00 18.06
ATOM    675  CB  PRO    89      17.733  12.294  46.494  1.00 17.00
ATOM    676  CG  PRO    89      17.726  11.261  47.607  1.00 15.83
ATOM    677  CD  PRO    89      18.844  11.642  48.560  1.00 17.16
ATOM    678  N   GLU    90      17.278  14.795  48.695  1.00 14.63
ATOM    679  CA  GLU    90      16.348  15.838  49.157  1.00 20.68
ATOM    680  C   GLU    90      16.701  17.229  48.645  1.00 25.39
ATOM    681  O   GLU    90      15.833  18.042  48.368  1.00 21.57
ATOM    682  CB  GLU    90      16.031  15.816  50.682  1.00 22.21
ATOM    683  CG  GLU    90      15.782  14.403  51.228  1.00 37.69
ATOM    684  CD  GLU    90      17.071  13.641  51.447  1.00 83.49
ATOM    685  OE1 GLU    90      18.179  14.151  51.342  1.00 54.80
ATOM    686  OE2 GLU    90      16.875  12.373  51.749  1.00 64.65
ATOM    687  N   GLY    91      17.977  17.509  48.510  1.00 21.39
ATOM    688  CA  GLY    91      18.394  18.769  47.906  1.00 17.77
ATOM    689  C   GLY    91      18.673  19.911  48.839  1.00 12.17
ATOM    690  O   GLY    91      18.769  19.764  50.055  1.00 16.81
ATOM    691  N   TYR    92      18.861  21.086  48.225  1.00 13.02
ATOM    692  CA  TYR    92      19.143  22.266  48.994  1.00 10.33
ATOM    693  C   TYR    92      18.575  23.478  48.347  1.00  9.87
ATOM    694  O   TYR    92      18.270  23.483  47.144  1.00 15.89
ATOM    695  CB  TYR    92      20.678  22.488  49.278  1.00 15.40
ATOM    696  CG  TYR    92      21.546  22.468  48.012  1.00 15.13
ATOM    697  CD1 TYR    92      21.620  23.576  47.166  1.00 14.75
ATOM    698  CD2 TYR    92      22.317  21.350  47.683  1.00 16.09
ATOM    699  CE1 TYR    92      22.404  23.561  46.008  1.00  6.50
ATOM    700  CE2 TYR    92      23.067  21.300  46.504  1.00 15.12
ATOM    701  CZ  TYR    92      23.156  22.424  45.683  1.00 18.13
ATOM    702  OH  TYR    92      23.944  22.393  44.517  1.00 13.37
ATOM    703  N   VAL    93      18.447  24.504  49.189  1.00 11.93
ATOM    704  CA  VAL    93      18.025  25.822  48.778  1.00 14.74
ATOM    705  C   VAL    93      19.281  26.666  48.625  1.00 16.00
ATOM    706  O   VAL    93      20.172  26.625  49.451  1.00 16.16
ATOM    707  CB  VAL    93      17.073  26.480  49.791  1.00 23.45
ATOM    708  CG1 VAL    93      16.855  27.937  49.413  1.00 26.05
ATOM    709  CG2 VAL    93      15.716  25.764  49.771  1.00 22.90
ATOM    710  N   GLN    94      19.361  27.345  47.521  1.00 13.78
ATOM    711  CA  GLN    94      20.480  28.195  47.227  1.00 10.53
ATOM    712  C   GLN    94      19.948  29.583  46.998  1.00 12.23
ATOM    713  O   GLN    94      19.153  29.788  46.061  1.00 15.52
ATOM    714  CB  GLN    94      21.232  27.727  45.934  1.00  7.95
ATOM    715  CG  GLN    94      22.361  28.708  45.469  1.00 11.87
ATOM    716  CD  GLN    94      23.431  27.999  44.632  1.00 12.04
ATOM    717  OE1 GLN    94      23.805  26.879  44.946  1.00 13.60
ATOM    718  NE2 GLN    94      23.719  28.527  43.449  1.00  7.98
ATOM    719  N   GLU    95      20.396  30.531  47.820  1.00 11.78
ATOM    720  CA  GLU    95      19.974  31.899  47.643  1.00 13.47
ATOM    721  C   GLU    95      21.149  32.804  47.398  1.00 18.42
ATOM    722  O   GLU    95      22.206  32.623  47.985  1.00 19.23
ATOM    723  CB  GLU    95      19.277  32.427  48.878  1.00 13.82
ATOM    724  CG  GLU    95      18.009  31.684  49.215  1.00 28.46
ATOM    725  CD  GLU    95      17.657  32.016  50.622  1.00 45.93
ATOM    726  OE1 GLU    95      17.574  33.166  51.011  1.00100.00
ATOM    727  OE2 GLU    95      17.764  30.987  51.423  1.00 61.33
ATOM    728  N   ARG    96      20.929  33.838  46.601  1.00 16.51
ATOM    729  CA  ARG    96      21.978  34.783  46.342  1.00 16.37
ATOM    730  C   ARG    96      21.510  36.195  46.206  1.00 15.84
```

FIG 5-12

ATOM	731	O	ARG	96	20.389	36.488	45.806	1.00	15.01
ATOM	732	CB	ARG	96	22.582	34.463	44.967	1.00	16.19
ATOM	733	CG	ARG	96	23.495	33.247	44.929	1.00	17.61
ATOM	734	CD	ARG	96	24.615	33.453	43.908	1.00	9.06
ATOM	735	NE	ARG	96	25.411	32.277	43.766	1.00	9.88
ATOM	736	CZ	ARG	96	25.434	31.493	42.693	1.00	20.03
ATOM	737	NH1	ARG	96	24.684	31.709	41.615	1.00	15.29
ATOM	738	NH2	ARG	96	26.236	30.430	42.714	1.00	11.03
ATOM	739	N	THR	97	22.470	37.068	46.344	1.00	13.39
ATOM	740	CA	THR	97	22.368	38.424	45.935	1.00	13.12
ATOM	741	C	THR	97	23.593	38.688	45.084	1.00	16.81
ATOM	742	O	THR	97	24.686	38.347	45.485	1.00	19.25
ATOM	743	CB	THR	97	22.282	39.442	47.066	1.00	26.27
ATOM	744	OG1	THR	97	21.225	39.101	47.945	1.00	31.43
ATOM	745	CG2	THR	97	22.038	40.804	46.445	1.00	15.90
ATOM	746	N	ILE	98	23.396	39.219	43.899	1.00	16.23
ATOM	747	CA	ILE	98	24.486	39.526	42.977	1.00	16.70
ATOM	748	C	ILE	98	24.533	41.017	42.686	1.00	21.10
ATOM	749	O	ILE	98	23.628	41.566	42.075	1.00	14.58
ATOM	750	CB	ILE	98	24.385	38.752	41.660	1.00	13.47
ATOM	751	CG1	ILE	98	24.480	37.236	41.890	1.00	16.09
ATOM	752	CG2	ILE	98	25.457	39.231	40.679	1.00	13.30
ATOM	753	CD1	ILE	98	23.875	36.431	40.738	1.00	13.93
ATOM	754	N	PHE	99	25.613	41.678	43.110	1.00	14.86
ATOM	755	CA	PHE	99	25.719	43.098	42.896	1.00	12.44
ATOM	756	C	PHE	99	26.514	43.441	41.699	1.00	20.37
ATOM	757	O	PHE	99	27.696	43.164	41.700	1.00	20.07
ATOM	758	CB	PHE	99	26.401	43.770	44.084	1.00	15.96
ATOM	759	CG	PHE	99	25.638	43.624	45.356	1.00	21.41
ATOM	760	CD1	PHE	99	25.863	42.524	46.189	1.00	24.98
ATOM	761	CD2	PHE	99	24.698	44.585	45.743	1.00	22.94
ATOM	762	CE1	PHE	99	25.176	42.400	47.400	1.00	32.06
ATOM	763	CE2	PHE	99	23.992	44.469	46.946	1.00	24.26
ATOM	764	CZ	PHE	99	24.235	43.369	47.771	1.00	28.19
ATOM	765	N	PHE	100	25.906	44.085	40.704	1.00	12.53
ATOM	766	CA	PHE	100	26.679	44.522	39.554	1.00	8.75
ATOM	767	C	PHE	100	27.294	45.855	39.872	1.00	21.81
ATOM	768	O	PHE	100	26.599	46.775	40.308	1.00	20.31
ATOM	769	CB	PHE	100	25.927	44.572	38.226	1.00	5.94
ATOM	770	CG	PHE	100	25.537	43.183	37.764	1.00	12.75
ATOM	771	CD1	PHE	100	24.426	42.538	38.325	1.00	16.31
ATOM	772	CD2	PHE	100	26.317	42.484	36.843	1.00	15.27
ATOM	773	CE1	PHE	100	24.087	41.230	37.975	1.00	13.50
ATOM	774	CE2	PHE	100	25.965	41.192	36.435	1.00	21.25
ATOM	775	CZ	PHE	100	24.852	40.567	37.014	1.00	21.06
ATOM	776	N	LYS	101	28.603	45.946	39.737	1.00	15.49
ATOM	777	CA	LYS	101	29.270	47.179	40.085	1.00	17.93
ATOM	778	C	LYS	101	28.732	48.349	39.287	1.00	13.71
ATOM	779	O	LYS	101	28.658	48.304	38.072	1.00	17.18
ATOM	780	CB	LYS	101	30.784	47.069	39.950	1.00	17.13
ATOM	781	CG	LYS	101	31.518	48.252	40.551	1.00	18.01
ATOM	782	CD	LYS	101	33.036	48.060	40.534	1.00	26.70
ATOM	783	CE	LYS	101	33.797	49.116	41.332	1.00	41.58
ATOM	784	N	ASP	102	28.353	49.403	39.997	1.00	18.09
ATOM	785	CA	ASP	102	27.805	50.618	39.368	1.00	23.08
ATOM	786	C	ASP	102	26.559	50.356	38.549	1.00	25.42
ATOM	787	O	ASP	102	26.292	51.061	37.586	1.00	23.34
ATOM	788	CB	ASP	102	28.840	51.369	38.516	1.00	26.27
ATOM	789	CG	ASP	102	30.109	51.629	39.296	1.00	57.01
ATOM	790	OD1	ASP	102	31.206	51.233	38.931	1.00	63.33
ATOM	791	OD2	ASP	102	29.896	52.200	40.464	1.00	47.66
ATOM	792	N	ASP	103	25.813	49.328	38.933	1.00	20.17
ATOM	793	CA	ASP	103	24.602	48.949	38.233	1.00	15.70
ATOM	794	C	ASP	103	23.608	48.284	39.189	1.00	18.47
ATOM	795	O	ASP	103	23.749	48.431	40.409	1.00	17.72
ATOM	796	CB	ASP	103	24.899	48.085	36.995	1.00	19.89
ATOM	797	CG	ASP	103	23.946	48.387	35.860	1.00	22.93

FIG 5-13

ATOM	798	OD1	ASP	103	24.238	48.274	34.688	1.00	19.05
ATOM	799	OD2	ASP	103	22.774	48.809	35.283	1.00	23.89
ATOM	800	N	GLY	104	22.612	47.542	38.646	1.00	20.17
ATOM	801	CA	GLY	104	21.598	46.900	39.498	1.00	20.22
ATOM	802	C	GLY	104	22.055	45.619	40.180	1.00	24.68
ATOM	803	O	GLY	104	23.202	45.211	40.085	1.00	18.06
ATOM	804	N	ASN	105	21.125	44.967	40.872	1.00	15.71
ATOM	805	CA	ASN	105	21.425	43.703	41.510	1.00	8.89
ATOM	806	C	ASN	105	20.399	42.620	41.181	1.00	21.85
ATOM	807	O	ASN	105	19.255	42.911	40.824	1.00	15.17
ATOM	808	CB	ASN	105	21.605	43.840	43.001	1.00	8.58
ATOM	809	CG	ASN	105	20.359	44.366	43.697	1.00	43.57
ATOM	810	OD1	ASN	105	19.565	43.601	44.259	1.00	36.67
ATOM	811	ND2	ASN	105	20.178	45.674	43.659	1.00	36.47
ATOM	812	N	TYR	106	20.826	41.365	41.328	1.00	16.80
ATOM	813	CA	TYR	106	19.966	40.219	41.156	1.00	13.90
ATOM	814	C	TYR	106	19.763	39.543	42.475	1.00	11.05
ATOM	815	O	TYR	106	20.678	39.404	43.281	1.00	13.86
ATOM	816	CB	TYR	106	20.547	39.128	40.246	1.00	15.88
ATOM	817	CG	TYR	106	20.619	39.398	38.793	1.00	15.57
ATOM	818	CD1	TYR	106	19.952	40.458	38.178	1.00	13.14
ATOM	819	CD2	TYR	106	21.373	38.524	38.006	1.00	13.35
ATOM	820	CE1	TYR	106	20.038	40.632	36.793	1.00	13.44
ATOM	821	CE2	TYR	106	21.481	38.692	36.628	1.00	10.87
ATOM	822	CZ	TYR	106	20.814	39.751	36.025	1.00	15.93
ATOM	823	OH	TYR	106	20.970	39.931	34.670	1.00	17.32
ATOM	824	N	LYS	107	18.538	39.115	42.709	1.00	12.39
ATOM	825	CA	LYS	107	18.194	38.349	43.897	1.00	11.51
ATOM	826	C	LYS	107	17.619	37.037	43.397	1.00	17.25
ATOM	827	O	LYS	107	16.704	37.010	42.562	1.00	13.14
ATOM	828	CB	LYS	107	17.217	39.063	44.823	1.00	14.82
ATOM	829	CG	LYS	107	17.860	39.631	46.060	1.00	40.71
ATOM	830	CD	LYS	107	18.528	40.974	45.793	1.00	43.48
ATOM	831	N	THR	108	18.205	35.951	43.835	1.00	14.95
ATOM	832	CA	THR	108	17.774	34.658	43.352	1.00	11.97
ATOM	833	C	THR	108	17.463	33.696	44.468	1.00	15.81
ATOM	834	O	THR	108	18.043	33.734	45.582	1.00	18.68
ATOM	835	CB	THR	108	18.847	34.034	42.410	1.00	23.81
ATOM	836	OG1	THR	108	20.064	33.791	43.137	1.00	13.88
ATOM	837	CG2	THR	108	19.123	34.968	41.264	1.00	13.04
ATOM	838	N	ARG	109	16.560	32.804	44.154	1.00	13.57
ATOM	839	CA	ARG	109	16.212	31.751	45.048	1.00	12.56
ATOM	840	C	ARG	109	15.939	30.498	44.254	1.00	13.07
ATOM	841	O	ARG	109	15.239	30.509	43.249	1.00	12.52
ATOM	842	CB	ARG	109	15.069	32.100	45.959	1.00	17.32
ATOM	843	CG	ARG	109	14.767	30.995	46.932	1.00	17.92
ATOM	844	CD	ARG	109	13.400	31.160	47.610	1.00	19.99
ATOM	845	NE	ARG	109	12.821	29.854	47.883	1.00	36.05
ATOM	846	CZ	ARG	109	12.968	29.244	49.035	1.00	55.71
ATOM	847	NH1	ARG	109	13.630	29.815	50.046	1.00	44.11
ATOM	848	NH2	ARG	109	12.432	28.041	49.195	1.00	94.34
ATOM	849	N	ALA	110	16.577	29.414	44.635	1.00	13.26
ATOM	850	CA	ALA	110	16.377	28.207	43.870	1.00	12.68
ATOM	851	C	ALA	110	16.346	26.979	44.734	1.00	13.15
ATOM	852	O	ALA	110	16.829	26.965	45.869	1.00	16.75
ATOM	853	CB	ALA	110	17.465	28.059	42.822	1.00	17.31
ATOM	854	N	GLU	111	15.770	25.939	44.176	1.00	15.39
ATOM	855	CA	GLU	111	15.741	24.655	44.823	1.00	15.24
ATOM	856	C	GLU	111	16.438	23.678	43.926	1.00	12.08
ATOM	857	O	GLU	111	16.086	23.545	42.771	1.00	15.70
ATOM	858	CB	GLU	111	14.303	24.123	44.993	1.00	19.20
ATOM	859	CG	GLU	111	13.744	24.242	46.399	1.00	38.62
ATOM	860	CD	GLU	111	12.247	24.280	46.372	1.00	60.99
ATOM	861	OE1	GLU	111	11.589	23.843	45.432	1.00	76.05
ATOM	862	OE2	GLU	111	11.742	24.956	47.280	1.00	54.87
ATOM	863	N	VAL	112	17.438	22.965	44.457	1.00	10.78
ATOM	864	CA	VAL	112	18.063	21.978	43.631	1.00	10.98

FIG 5-14

ATOM	865	C	VAL	112	17.968	20.630	44.261	1.00	8.62
ATOM	866	O	VAL	112	18.271	20.438	45.432	1.00	15.63
ATOM	867	CB	VAL	112	19.428	22.358	43.012	1.00	22.75
ATOM	868	CG1	VAL	112	19.966	23.704	43.487	1.00	16.69
ATOM	869	CG2	VAL	112	20.452	21.232	43.078	1.00	18.47
ATOM	870	N	LYS	113	17.415	19.732	43.516	1.00	14.67
ATOM	871	CA	LYS	113	17.175	18.421	44.045	1.00	16.41
ATOM	872	C	LYS	113	16.822	17.485	42.931	1.00	7.11
ATOM	873	O	LYS	113	16.695	17.893	41.808	1.00	16.27
ATOM	874	CB	LYS	113	16.032	18.497	45.036	1.00	22.50
ATOM	875	CG	LYS	113	14.792	19.084	44.376	1.00	20.40
ATOM	876	CD	LYS	113	13.509	18.321	44.703	1.00	44.65
ATOM	877	CE	LYS	113	12.526	19.134	45.528	1.00	54.02
ATOM	878	NZ	LYS	113	12.379	20.518	45.036	1.00	100.00
ATOM	879	N	PHE	114	16.683	16.208	43.267	1.00	10.09
ATOM	880	CA	PHE	114	16.325	15.175	42.317	1.00	11.41
ATOM	881	C	PHE	114	14.806	14.975	42.181	1.00	14.18
ATOM	882	O	PHE	114	14.110	14.878	43.160	1.00	15.03
ATOM	883	CB	PHE	114	16.866	13.838	42.838	1.00	12.89
ATOM	884	CG	PHE	114	18.231	13.536	42.338	1.00	16.80
ATOM	885	CD1	PHE	114	19.344	13.795	43.139	1.00	18.61
ATOM	886	CD2	PHE	114	18.403	13.009	41.056	1.00	19.50
ATOM	887	CE1	PHE	114	20.627	13.500	42.665	1.00	22.78
ATOM	888	CE2	PHE	114	19.673	12.708	40.572	1.00	25.36
ATOM	889	CZ	PHE	114	20.780	12.953	41.387	1.00	23.99
ATOM	890	N	GLU	115	14.354	14.819	40.966	1.00	15.29
ATOM	891	CA	GLU	115	12.978	14.473	40.642	1.00	11.40
ATOM	892	C	GLU	115	13.121	13.193	39.906	1.00	13.30
ATOM	893	O	GLU	115	13.434	13.207	38.730	1.00	18.72
ATOM	894	CB	GLU	115	12.348	15.481	39.667	1.00	9.68
ATOM	895	CG	GLU	115	11.856	16.747	40.376	1.00	19.54
ATOM	896	CD	GLU	115	10.742	16.460	41.342	1.00	38.12
ATOM	897	OE1	GLU	115	10.181	15.395	41.431	1.00	34.84
ATOM	898	OE2	GLU	115	10.460	17.461	42.079	1.00	27.88
ATOM	899	N	GLY	116	13.005	12.087	40.585	1.00	14.51
ATOM	900	CA	GLY	116	13.225	10.861	39.869	1.00	15.91
ATOM	901	C	GLY	116	14.727	10.767	39.641	1.00	23.59
ATOM	902	O	GLY	116	15.516	10.922	40.570	1.00	19.35
ATOM	903	N	ASP	117	15.137	10.564	38.439	1.00	20.26
ATOM	904	CA	ASP	117	16.572	10.462	38.233	1.00	28.00
ATOM	905	C	ASP	117	17.237	11.677	37.598	1.00	22.39
ATOM	906	O	ASP	117	18.423	11.672	37.265	1.00	21.38
ATOM	907	CB	ASP	117	17.055	9.074	37.733	1.00	33.06
ATOM	908	CG	ASP	117	16.624	8.677	36.348	1.00	55.04
ATOM	909	OD1	ASP	117	16.230	9.468	35.495	1.00	59.57
ATOM	910	OD2	ASP	117	16.805	7.391	36.130	1.00	82.48
ATOM	911	N	THR	118	16.463	12.729	37.493	1.00	19.62
ATOM	912	CA	THR	118	16.889	13.981	36.910	1.00	18.21
ATOM	913	C	THR	118	17.186	14.988	37.976	1.00	18.92
ATOM	914	O	THR	118	16.498	15.064	38.996	1.00	15.94
ATOM	915	CB	THR	118	15.806	14.497	35.952	1.00	19.03
ATOM	916	OG1	THR	118	15.552	13.508	34.990	1.00	21.42
ATOM	917	CG2	THR	118	16.217	15.793	35.275	1.00	15.49
ATOM	918	N	LEU	119	18.284	15.681	37.805	1.00	13.66
ATOM	919	CA	LEU	119	18.679	16.706	38.759	1.00	13.50
ATOM	920	C	LEU	119	18.036	17.992	38.269	1.00	8.81
ATOM	921	O	LEU	119	18.194	18.368	37.091	1.00	12.49
ATOM	922	CB	LEU	119	20.243	16.815	38.839	1.00	12.25
ATOM	923	CG	LEU	119	20.845	17.678	39.951	1.00	3.90
ATOM	924	CD1	LEU	119	20.701	19.167	39.669	1.00	10.11
ATOM	925	CD2	LEU	119	20.366	17.311	41.333	1.00	7.36
ATOM	926	N	VAL	120	17.230	13.595	39.170	1.00	13.34
ATOM	927	CA	VAL	120	16.466	19.797	38.859	1.00	13.77
ATOM	928	C	VAL	120	16.929	21.039	39.587	1.00	8.56
ATOM	929	O	VAL	120	17.135	21.039	40.762	1.00	13.32
ATOM	930	CB	VAL	120	14.939	19.566	39.082	1.00	17.60
ATOM	931	CG1	VAL	120	14.133	20.790	38.642	1.00	17.38

FIG 5-15

ATOM	932	CG2	VAL	120	14.501	18.351	38.246	1.00	15.35
ATOM	933	N	ASN	121	17.067	22.111	38.839	1.00	12.24
ATOM	934	CA	ASN	121	17.424	23.405	39.400	1.00	11.78
ATOM	935	C	ASN	121	16.301	24.382	39.060	1.00	11.18
ATOM	936	O	ASN	121	16.195	24.802	37.934	1.00	11.09
ATOM	937	CB	ASN	121	18.753	23.928	38.791	1.00	11.41
ATOM	938	CG	ASN	121	19.201	25.261	39.367	1.00	11.07
ATOM	939	OD1	ASN	121	18.773	25.654	40.461	1.00	12.06
ATOM	940	ND2	ASN	121	20.124	25.938	38.670	1.00	11.90
ATOM	941	N	ARG	122	15.470	24.706	40.029	1.00	13.69
ATOM	942	CA	ARG	122	14.348	25.610	39.825	1.00	12.99
ATOM	943	C	ARG	122	14.622	26.946	40.498	1.00	5.89
ATOM	944	O	ARG	122	14.749	27.011	41.723	1.00	14.47
ATOM	945	CB	ARG	122	13.068	25.025	40.417	1.00	15.99
ATOM	946	CG	ARG	122	12.478	23.921	39.589	1.00	30.23
ATOM	947	CD	ARG	122	11.282	23.244	40.281	1.00	60.61
ATOM	948	N	ILE	123	14.663	27.992	39.680	1.00	11.46
ATOM	949	CA	ILE	123	15.030	29.340	40.095	1.00	11.86
ATOM	950	C	ILE	123	13.991	30.450	39.835	1.00	10.54
ATOM	951	O	ILE	123	13.370	30.535	38.765	1.00	12.83
ATOM	952	CB	ILE	123	16.296	29.757	39.292	1.00	15.41
ATOM	953	CG1	ILE	123	17.316	28.585	39.180	1.00	12.27
ATOM	954	CG2	ILE	123	16.944	30.993	39.918	1.00	14.01
ATOM	955	CD1	ILE	123	17.652	28.242	37.743	1.00	7.74
ATOM	956	N	GLU	124	13.953	31.358	40.793	1.00	11.36
ATOM	957	CA	GLU	124	13.189	32.572	40.700	1.00	15.20
ATOM	958	C	GLU	124	14.168	33.713	40.811	1.00	11.93
ATOM	959	O	GLU	124	14.919	33.797	41.780	1.00	15.61
ATOM	960	CB	GLU	124	12.028	32.677	41.751	1.00	19.74
ATOM	961	CG	GLU	124	12.387	33.337	43.089	1.00	72.94
ATOM	962	N	LEU	125	14.183	34.550	39.808	1.00	12.19
ATOM	963	CA	LEU	125	15.092	35.654	39.767	1.00	15.00
ATOM	964	C	LEU	125	14.420	37.011	39.722	1.00	19.35
ATOM	965	O	LEU	125	13.563	37.267	38.893	1.00	18.41
ATOM	966	CB	LEU	125	15.976	35.533	38.510	1.00	14.29
ATOM	967	CG	LEU	125	17.003	36.683	38.375	1.00	17.65
ATOM	968	CD1	LEU	125	18.302	36.083	37.849	1.00	13.46
ATOM	969	CD2	LEU	125	16.511	37.732	37.367	1.00	12.09
ATOM	970	N	LYS	126	14.890	37.897	40.554	1.00	12.73
ATOM	971	CA	LYS	126	14.391	39.260	40.579	1.00	15.92
ATOM	972	C	LYS	126	15.563	40.276	40.445	1.00	18.53
ATOM	973	O	LYS	126	16.489	40.246	41.246	1.00	19.86
ATOM	974	CB	LYS	126	13.611	39.487	41.877	1.00	17.31
ATOM	975	CG	LYS	126	12.853	40.786	41.923	1.00	33.94
ATOM	976	CD	LYS	126	11.366	40.601	41.675	1.00	60.87
ATOM	977	CE	LYS	126	10.652	41.929	41.521	1.00	52.70
ATOM	978	NZ	LYS	126	11.229	42.988	42.367	1.00	47.22
ATOM	979	N	GLY	127	15.514	41.127	39.411	1.00	18.71
ATOM	980	CA	GLY	127	16.551	42.151	39.121	1.00	17.32
ATOM	981	C	GLY	127	16.012	43.572	39.272	1.00	25.32
ATOM	982	O	GLY	127	14.981	43.908	38.693	1.00	20.14
ATOM	983	N	ILE	128	16.706	44.404	40.070	1.00	18.42
ATOM	984	CA	ILE	128	16.282	45.787	40.243	1.00	21.04
ATOM	985	C	ILE	128	17.405	46.789	40.196	1.00	25.93
ATOM	986	O	ILE	128	18.562	46.496	40.429	1.00	19.37
ATOM	987	CB	ILE	128	15.482	46.052	41.504	1.00	23.82
ATOM	988	CG1	ILE	128	16.408	45.888	42.701	1.00	25.86
ATOM	989	CG2	ILE	128	14.272	45.120	41.577	1.00	28.95
ATOM	990	CD1	ILE	128	15.824	46.391	44.013	1.00	29.89
ATOM	991	N	ASP	129	16.999	48.002	39.918	1.00	20.26
ATOM	992	CA	ASP	129	17.861	49.124	39.882	1.00	18.53
ATOM	993	C	ASP	129	18.864	49.086	38.801	1.00	20.36
ATOM	994	O	ASP	129	19.949	49.632	38.953	1.00	24.28
ATOM	995	CB	ASP	129	18.498	49.407	41.253	1.00	20.57
ATOM	996	CG	ASP	129	17.545	50.077	42.226	1.00	43.70
ATOM	997	OD1	ASP	129	16.653	50.842	41.883	1.00	49.42
ATOM	998	OD2	ASP	129	17.770	49.740	43.475	1.00	38.07

FIG 5-16

ATOM	999	N	PHE	130	18.510	48.493	37.693	1.00	16.40
ATOM	1000	CA	PHE	130	19.433	48.459	36.563	1.00	16.99
ATOM	1001	C	PHE	130	19.330	49.732	35.756	1.00	35.37
ATOM	1002	O	PHE	130	18.242	50.318	35.623	1.00	27.34
ATOM	1003	CB	PHE	130	19.248	47.223	35.657	1.00	18.07
ATOM	1004	CG	PHE	130	19.809	45.980	36.312	1.00	19.10
ATOM	1005	CD1	PHE	130	19.021	45.210	37.171	1.00	16.15
ATOM	1006	CD2	PHE	130	21.126	45.572	36.073	1.00	19.17
ATOM	1007	CE1	PHE	130	19.536	44.074	37.801	1.00	23.37
ATOM	1008	CE2	PHE	130	21.665	44.445	36.703	1.00	21.11
ATOM	1009	CZ	PHE	130	20.867	43.703	37.575	1.00	22.13
ATOM	1010	N	LYS	131	20.464	50.169	35.218	1.00	31.09
ATOM	1011	CA	LYS	131	20.477	51.371	34.400	1.00	27.52
ATOM	1012	C	LYS	131	20.105	51.045	32.992	1.00	25.57
ATOM	1013	O	LYS	131	20.695	50.169	32.343	1.00	22.97
ATOM	1014	CB	LYS	131	21.796	52.109	34.438	1.00	32.64
ATOM	1015	CG	LYS	131	22.153	52.633	35.813	1.00	38.34
ATOM	1016	CD	LYS	131	23.646	52.886	35.975	1.00	75.76
ATOM	1017	N	GLU	132	19.116	51.751	32.509	1.00	26.88
ATOM	1018	CA	GLU	132	18.623	51.484	31.189	1.00	28.42
ATOM	1019	C	GLU	132	19.710	51.514	30.140	1.00	36.19
ATOM	1020	O	GLU	132	19.617	50.862	29.101	1.00	39.24
ATOM	1021	CB	GLU	132	17.374	52.331	30.830	1.00	29.04
ATOM	1022	N	ASP	133	20.752	52.254	30.438	1.00	40.08
ATOM	1023	CA	ASP	133	21.883	52.442	29.525	1.00	45.36
ATOM	1024	C	ASP	133	23.224	51.861	30.049	1.00	50.61
ATOM	1025	O	ASP	133	24.299	52.243	29.572	1.00	52.14
ATOM	1026	CB	ASP	133	22.063	53.946	29.332	1.00	50.45
ATOM	1027	CG	ASP	133	22.109	54.642	30.670	1.00	87.10
ATOM	1028	OD1	ASP	133	21.408	54.314	31.624	1.00	91.27
ATOM	1029	OD2	ASP	133	23.047	55.552	30.739	1.00	100.00
ATOM	1030	N	GLY	134	23.159	50.970	31.053	1.00	37.06
ATOM	1031	CA	GLY	134	24.349	50.376	31.639	1.00	30.22
ATOM	1032	C	GLY	134	24.845	49.228	30.803	1.00	23.10
ATOM	1033	O	GLY	134	24.360	48.990	29.685	1.00	19.23
ATOM	1034	N	ASN	135	25.807	48.486	31.341	1.00	18.66
ATOM	1035	CA	ASN	135	26.339	47.370	30.563	1.00	18.03
ATOM	1036	C	ASN	135	25.372	46.199	30.406	1.00	15.75
ATOM	1037	O	ASN	135	25.485	45.430	29.461	1.00	16.03
ATOM	1038	CB	ASN	135	27.665	46.883	31.139	1.00	19.27
ATOM	1039	CG	ASN	135	28.743	47.943	31.108	1.00	20.99
ATOM	1040	OD1	ASN	135	28.969	48.595	30.078	1.00	25.69
ATOM	1041	ND2	ASN	135	29.423	48.095	32.239	1.00	22.57
ATOM	1042	N	ILE	136	24.444	46.052	31.362	1.00	18.14
ATOM	1043	CA	ILE	136	23.494	44.924	31.368	1.00	19.78
ATOM	1044	C	ILE	136	22.331	45.086	30.384	1.00	23.76
ATOM	1045	O	ILE	136	22.178	44.313	29.395	1.00	22.53
ATOM	1046	CB	ILE	136	23.078	44.500	32.804	1.00	21.24
ATOM	1047	CG1	ILE	136	24.230	43.728	33.423	1.00	28.44
ATOM	1048	CG2	ILE	136	21.899	43.543	32.770	1.00	22.77
ATOM	1049	CD1	ILE	136	25.346	44.596	33.935	1.00	12.39
ATOM	1050	N	LEU	137	21.543	46.117	30.640	1.00	18.21
ATOM	1051	CA	LEU	137	20.394	46.415	29.815	1.00	23.30
ATOM	1052	C	LEU	137	20.828	46.875	28.470	1.00	27.26
ATOM	1053	O	LEU	137	20.181	46.619	27.488	1.00	27.00
ATOM	1054	CB	LEU	137	19.442	47.430	30.490	1.00	21.74
ATOM	1055	CG	LEU	137	18.828	46.852	31.762	1.00	22.56
ATOM	1056	CD1	LEU	137	17.856	47.837	32.415	1.00	22.27
ATOM	1057	CD2	LEU	137	18.118	45.554	31.424	1.00	37.52
ATOM	1058	N	GLY	138	21.979	47.527	28.432	1.00	22.14
ATOM	1059	CA	GLY	138	22.510	48.033	27.187	1.00	20.03
ATOM	1060	C	GLY	138	23.157	46.959	26.368	1.00	20.16
ATOM	1061	O	GLY	138	23.600	47.202	25.264	1.00	22.44
ATOM	1062	N	HIS	139	23.246	45.786	26.903	1.00	18.27
ATOM	1063	CA	HIS	139	23.859	44.685	26.148	1.00	20.24
ATOM	1064	C	HIS	139	25.301	44.929	25.616	1.00	20.13
ATOM	1065	O	HIS	139	25.605	44.745	24.439	1.00	17.97

FIG 5-17

ATOM	1066	CB	HIS	139	22.931	44.207	25.018	1.00	22.20
ATOM	1067	CG	HIS	139	21.708	43.551	25.550	1.00	25.52
ATOM	1068	ND1	HIS	139	21.666	42.182	25.785	1.00	25.67
ATOM	1069	CD2	HIS	139	20.525	44.092	25.927	1.00	28.09
ATOM	1070	CE1	HIS	139	20.474	41.918	26.275	1.00	27.50
ATOM	1071	NE2	HIS	139	19.766	43.044	26.382	1.00	29.53
ATOM	1072	N	LYS	140	26.187	45.311	26.525	1.00	23.51
ATOM	1073	CA	LYS	140	27.569	45.638	26.197	1.00	25.82
ATOM	1074	C	LYS	140	28.600	44.537	26.560	1.00	26.28
ATOM	1075	O	LYS	140	29.824	44.730	26.391	1.00	22.29
ATOM	1076	CB	LYS	140	27.977	46.937	26.911	1.00	27.56
ATOM	1077	CG	LYS	140	27.269	48.217	26.445	1.00	31.19
ATOM	1078	CD	LYS	140	27.234	49.254	27.582	1.00	51.32
ATOM	1079	CE	LYS	140	26.924	50.696	27.169	1.00	47.92
ATOM	1080	NZ	LYS	140	27.112	51.663	28.284	1.00	73.76
ATOM	1081	N	LEU	141	28.116	43.403	27.115	1.00	19.33
ATOM	1082	CA	LEU	141	28.987	42.296	27.559	1.00	14.32
ATOM	1083	C	LEU	141	29.366	41.401	26.427	1.00	20.75
ATOM	1084	O	LEU	141	28.526	41.087	25.620	1.00	19.01
ATOM	1085	CB	LEU	141	28.313	41.488	28.676	1.00	12.53
ATOM	1086	CG	LEU	141	27.979	42.352	29.875	1.00	17.54
ATOM	1087	CD1	LEU	141	27.700	41.469	31.070	1.00	24.81
ATOM	1088	CD2	LEU	141	29.116	43.310	30.182	1.00	27.50
ATOM	1089	N	GLU	142	30.644	40.987	26.346	1.00	14.76
ATOM	1090	CA	GLU	142	31.040	40.059	25.311	1.00	13.43
ATOM	1091	C	GLU	142	30.462	38.691	25.641	1.00	15.69
ATOM	1092	O	GLU	142	30.175	38.393	26.787	1.00	16.43
ATOM	1093	CB	GLU	142	32.558	39.866	25.204	1.00	14.73
ATOM	1094	CG	GLU	142	33.290	41.077	24.624	1.00	29.30
ATOM	1095	CD	GLU	142	34.787	41.003	24.825	1.00	56.32
ATOM	1096	OE1	GLU	142	35.340	40.098	25.420	1.00	31.70
ATOM	1097	OE2	GLU	142	35.430	42.015	24.321	1.00	34.10
ATOM	1098	N	TYR	143	30.365	37.873	24.632	1.00	16.30
ATOM	1099	CA	TYR	143	29.837	36.542	24.764	1.00	20.04
ATOM	1100	C	TYR	143	30.925	35.559	25.049	1.00	12.46
ATOM	1101	O	TYR	143	31.327	34.792	24.193	1.00	16.99
ATOM	1102	CB	TYR	143	29.035	36.118	23.498	1.00	20.96
ATOM	1103	CG	TYR	143	28.187	34.857	23.674	1.00	16.12
ATOM	1104	CD1	TYR	143	27.040	34.859	24.472	1.00	18.24
ATOM	1105	CD2	TYR	143	28.512	33.684	22.986	1.00	12.87
ATOM	1106	CE1	TYR	143	26.257	33.708	24.615	1.00	17.91
ATOM	1107	CE2	TYR	143	27.735	32.530	23.104	1.00	16.58
ATOM	1108	CZ	TYR	143	26.603	32.551	23.914	1.00	17.35
ATOM	1109	OH	TYR	143	25.861	31.432	24.035	1.00	23.40
ATOM	1110	N	ASN	144	31.392	35.597	26.251	1.00	12.40
ATOM	1111	CA	ASN	144	32.428	34.703	26.689	1.00	12.05
ATOM	1112	C	ASN	144	32.433	34.675	28.193	1.00	15.75
ATOM	1113	O	ASN	144	31.637	35.369	28.837	1.00	14.58
ATOM	1114	CB	ASN	144	33.823	35.038	26.068	1.00	18.45
ATOM	1115	CG	ASN	144	34.310	36.445	26.374	1.00	18.98
ATOM	1116	OD1	ASN	144	34.150	36.951	27.488	1.00	20.34
ATOM	1117	ND2	ASN	144	34.891	37.085	25.382	1.00	23.02
ATOM	1118	N	TYR	145	33.311	33.876	28.773	1.00	12.16
ATOM	1119	CA	TYR	145	33.343	33.765	30.195	1.00	10.63
ATOM	1120	C	TYR	145	34.765	33.458	30.730	1.00	14.58
ATOM	1121	O	TYR	145	35.510	32.751	30.090	1.00	18.83
ATOM	1122	CB	TYR	145	32.404	32.627	30.571	1.00	9.76
ATOM	1123	CG	TYR	145	31.698	32.916	31.826	1.00	11.86
ATOM	1124	CD1	TYR	145	30.515	33.658	31.808	1.00	9.04
ATOM	1125	CD2	TYR	145	32.188	32.419	33.030	1.00	10.07
ATOM	1126	CE1	TYR	145	29.860	33.948	32.999	1.00	8.36
ATOM	1127	CE2	TYR	145	31.544	32.707	34.235	1.00	15.32
ATOM	1128	CZ	TYR	145	30.375	33.469	34.206	1.00	11.69
ATOM	1129	OH	TYR	145	29.730	33.735	35.376	1.00	15.23
ATOM	1130	N	ASN	146	35.086	33.931	31.933	1.00	15.36
ATOM	1131	CA	ASN	146	36.415	33.737	32.560	1.00	17.00
ATOM	1132	C	ASN	146	36.426	32.618	33.589	1.00	19.68

FIG 5-18

```
ATOM   1133  O   ASN  146      35.395  32.043  33.848  1.00 14.71
ATOM   1134  CB  ASN  146      36.844  35.062  33.235  1.00 11.89
ATOM   1135  CG  ASN  146      37.013  36.147  32.215  1.00 35.45
ATOM   1136  OD1 ASN  146      37.533  35.890  31.105  1.00 31.63
ATOM   1137  ND2 ASN  146      36.547  37.349  32.553  1.00 19.74
ATOM   1138  N   SER  147      37.630  32.338  34.201  1.00 12.09
ATOM   1139  CA  SER  147      37.804  31.320  35.266  1.00  8.55
ATOM   1140  C   SER  147      37.769  31.999  36.575  1.00 11.70
ATOM   1141  O   SER  147      38.219  33.125  36.671  1.00 16.56
ATOM   1142  CB  SER  147      39.148  30.540  35.129  1.00  9.87
ATOM   1143  OG  SER  147      39.212  29.980  33.828  1.00 33.20
ATOM   1144  N   HIS  148      37.195  31.365  37.583  1.00  5.53
ATOM   1145  CA  HIS  148      37.090  31.998  38.850  1.00  8.06
ATOM   1146  C   HIS  148      37.346  31.038  39.949  1.00 11.30
ATOM   1147  O   HIS  148      37.328  29.844  39.754  1.00 16.87
ATOM   1148  CB  HIS  148      35.648  32.608  39.067  1.00 11.29
ATOM   1149  CG  HIS  148      35.215  33.554  37.972  1.00 10.84
ATOM   1150  ND1 HIS  148      34.548  33.121  36.836  1.00 12.77
ATOM   1151  CD2 HIS  148      35.403  34.887  37.851  1.00  8.82
ATOM   1152  CE1 HIS  148      34.389  34.178  36.060  1.00  8.84
ATOM   1153  NE2 HIS  148      34.882  35.242  36.647  1.00  8.82
ATOM   1154  N   ASN  149      37.534  31.579  41.125  1.00 10.80
ATOM   1155  CA  ASN  149      37.626  30.805  42.345  1.00 13.35
ATOM   1156  C   ASN  149      36.409  31.157  43.205  1.00 14.47
ATOM   1157  O   ASN  149      36.099  32.320  43.387  1.00 18.17
ATOM   1158  CB  ASN  149      38.890  31.093  43.184  1.00 12.67
ATOM   1159  CG  ASN  149      40.148  30.822  42.424  1.00 20.21
ATOM   1160  OD1 ASN  149      40.993  31.713  42.281  1.00 56.34
ATOM   1161  ND2 ASN  149      40.210  29.641  41.818  1.00 16.44
ATOM   1162  N   VAL  150      35.773  30.144  43.741  1.00 14.65
ATOM   1163  CA  VAL  150      34.588  30.262  44.552  1.00 12.92
ATOM   1164  C   VAL  150      34.910  29.806  45.943  1.00 16.30
ATOM   1165  O   VAL  150      35.257  28.665  46.147  1.00 17.83
ATOM   1166  CB  VAL  150      33.482  29.382  43.914  1.00 15.22
ATOM   1167  CG1 VAL  150      32.252  29.297  44.765  1.00 14.09
ATOM   1168  CG2 VAL  150      33.172  29.791  42.464  1.00 10.94
ATOM   1169  N   TYR  151      34.796  30.716  46.900  1.00 17.64
ATOM   1170  CA  TYR  151      35.139  30.440  48.275  1.00 18.31
ATOM   1171  C   TYR  151      34.003  29.917  49.117  1.00 24.35
ATOM   1172  O   TYR  151      32.963  30.536  49.239  1.00 20.83
ATOM   1173  CB  TYR  151      35.793  31.681  48.920  1.00 20.15
ATOM   1174  CG  TYR  151      37.025  32.033  48.141  1.00 25.86
ATOM   1175  CD1 TYR  151      37.003  32.989  47.127  1.00 26.00
ATOM   1176  CD2 TYR  151      38.200  31.315  48.355  1.00 28.66
ATOM   1177  CE1 TYR  151      38.151  33.234  46.369  1.00 33.73
ATOM   1178  CE2 TYR  151      39.360  31.550  47.619  1.00 29.01
ATOM   1179  CZ  TYR  151      39.325  32.512  46.618  1.00 29.55
ATOM   1180  OH  TYR  151      40.449  32.737  45.877  1.00 38.69
ATOM   1181  N   ILE  152      34.250  28.791  49.753  1.00 17.71
ATOM   1182  CA  ILE  152      33.255  28.159  50.572  1.00 14.12
ATOM   1183  C   ILE  152      33.619  28.056  52.000  1.00 18.51
ATOM   1184  O   ILE  152      34.728  27.703  52.336  1.00 22.05
ATOM   1185  CB  ILE  152      32.979  26.776  50.060  1.00 16.66
ATOM   1186  CG1 ILE  152      32.431  26.875  48.638  1.00 11.30
ATOM   1187  CG2 ILE  152      32.017  26.078  51.021  1.00 17.96
ATOM   1188  CD1 ILE  152      32.377  25.559  47.949  1.00 13.48
ATOM   1189  N   MSE  153      32.623  28.278  52.841  1.00 17.41
ATOM   1190  CA  MSE  153      32.789  28.162  54.269  1.00 22.61
ATOM   1191  C   MSE  153      31.534  27.648  54.916  1.00 27.31
ATOM   1192  O   MSE  153      30.433  27.831  54.396  1.00 20.50
ATOM   1193  CB  MSE  153      33.145  29.490  54.855  1.00 19.11
ATOM   1194  CG  MSE  153      34.010  30.302  53.957  1.00100.00
ATOM   1195  SE  MSE  153      34.060  32.117  54.524  1.00100.00
ATOM   1196  CE  MSE  153      33.463  31.798  56.330  1.00 30.27
ATOM   1197  N   ALA  154      31.733  26.983  56.053  1.00 22.29
ATOM   1198  CA  ALA  154      30.669  26.389  56.796  1.00 22.66
ATOM   1199  C   ALA  154      29.820  27.401  57.552  1.00 29.00
```

FIG 5-19

ATOM	1200	O	ALA	154	30.274	28.457	57.960	1.00	27.02
ATOM	1201	CB	ALA	154	31.224	25.336	57.744	1.00	19.78
ATOM	1202	N	ASP	155	28.566	27.063	57.726	1.00	29.48
ATOM	1203	CA	ASP	155	27.669	27.887	58.484	1.00	32.18
ATOM	1204	C	ASP	155	26.976	27.019	59.511	1.00	44.81
ATOM	1205	O	ASP	155	25.898	26.492	59.274	1.00	39.56
ATOM	1206	CB	ASP	155	26.659	28.617	57.597	1.00	31.70
ATOM	1207	CG	ASP	155	26.140	29.851	58.247	1.00	49.89
ATOM	1208	OD1	ASP	155	26.595	30.297	59.277	1.00	46.67
ATOM	1209	OD2	ASP	155	25.187	30.422	57.565	1.00	76.07
ATOM	1210	N	LYS	156	27.646	26.816	60.629	1.00	46.37
ATOM	1211	CA	LYS	156	27.116	25.954	61.654	1.00	53.23
ATOM	1212	C	LYS	156	25.750	26.369	62.224	1.00	65.62
ATOM	1213	O	LYS	156	25.012	25.520	62.703	1.00	65.54
ATOM	1214	CB	LYS	156	28.147	25.612	62.725	1.00	59.51
ATOM	1215	N	GLN	157	25.398	27.655	62.138	1.00	68.32
ATOM	1216	CA	GLN	157	24.119	28.135	62.670	1.00	73.00
ATOM	1217	C	GLN	157	22.891	27.767	61.817	1.00	87.53
ATOM	1218	O	GLN	157	21.778	27.547	62.325	1.00	96.16
ATOM	1219	N	LYS	158	23.095	27.725	60.506	1.00	72.49
ATOM	1220	CA	LYS	158	22.040	27.386	59.593	1.00	66.19
ATOM	1221	C	LYS	158	22.235	25.985	59.040	1.00	58.21
ATOM	1222	O	LYS	158	21.447	25.524	58.226	1.00	59.85
ATOM	1223	N	ASN	159	23.303	25.294	59.502	1.00	40.00
ATOM	1224	CA	ASN	159	23.582	23.944	59.012	1.00	36.67
ATOM	1225	C	ASN	159	23.755	24.002	57.500	1.00	34.11
ATOM	1226	O	ASN	159	23.223	23.167	56.754	1.00	31.69
ATOM	1227	CB	ASN	159	22.431	22.952	59.367	1.00	46.42
ATOM	1228	CG	ASN	159	22.842	21.485	59.428	1.00	80.46
ATOM	1229	OD1	ASN	159	23.850	21.121	60.054	1.00	100.00
ATOM	1230	ND2	ASN	159	22.003	20.620	58.854	1.00	58.09
ATOM	1231	N	GLY	160	24.474	25.044	57.062	1.00	22.34
ATOM	1232	CA	GLY	160	24.686	25.247	55.663	1.00	17.58
ATOM	1233	C	GLY	160	26.055	25.791	55.433	1.00	26.75
ATOM	1234	O	GLY	160	26.960	25.664	56.271	1.00	25.57
ATOM	1235	N	ILE	161	26.200	26.395	54.277	1.00	23.28
ATOM	1236	CA	ILE	161	27.442	26.975	53.909	1.00	16.45
ATOM	1237	C	ILE	161	27.200	28.354	53.395	1.00	15.77
ATOM	1238	O	ILE	161	26.118	28.680	52.962	1.00	15.95
ATOM	1239	CB	ILE	161	28.129	26.117	52.864	1.00	19.27
ATOM	1240	CG1	ILE	161	27.237	26.016	51.619	1.00	18.53
ATOM	1241	CG2	ILE	161	28.351	24.735	53.445	1.00	21.96
ATOM	1242	CD1	ILE	161	28.009	25.614	50.350	1.00	14.44
ATOM	1243	N	LYS	162	28.226	29.169	53.471	1.00	17.96
ATOM	1244	CA	LYS	162	28.187	30.508	52.948	1.00	14.42
ATOM	1245	C	LYS	162	29.216	30.524	51.857	1.00	17.73
ATOM	1246	O	LYS	162	30.249	29.875	51.991	1.00	19.16
ATOM	1247	CB	LYS	162	28.480	31.540	54.055	1.00	18.15
ATOM	1248	CG	LYS	162	27.221	31.963	54.796	1.00	42.68
ATOM	1249	CD	LYS	162	27.493	32.787	56.039	1.00	70.42
ATOM	1250	N	VAL	163	28.911	31.176	50.759	1.00	13.74
ATOM	1251	CA	VAL	163	29.798	31.201	49.629	1.00	11.95
ATOM	1252	C	VAL	163	29.928	32.610	49.103	1.00	19.30
ATOM	1253	O	VAL	163	28.944	33.318	48.983	1.00	19.84
ATOM	1254	CB	VAL	163	29.249	30.268	48.532	1.00	15.89
ATOM	1255	CG1	VAL	163	30.105	30.277	47.261	1.00	12.09
ATOM	1256	CG2	VAL	163	29.029	28.852	49.077	1.00	15.88
ATOM	1257	N	ASN	164	31.146	32.999	48.733	1.00	14.03
ATOM	1258	CA	ASN	164	31.382	34.310	48.195	1.00	15.58
ATOM	1259	C	ASN	164	32.396	34.271	47.050	1.00	20.08
ATOM	1260	O	ASN	164	33.268	33.386	46.988	1.00	23.49
ATOM	1261	CB	ASN	164	31.732	35.325	49.308	1.00	20.52
ATOM	1262	CG	ASN	164	33.196	35.697	49.330	1.00	89.21
ATOM	1263	OD1	ASN	164	34.020	34.987	49.929	1.00	100.00
ATOM	1264	ND2	ASN	164	33.515	36.831	48.700	1.00	91.46
ATOM	1265	N	PHE	165	32.244	35.207	46.109	1.00	17.37
ATOM	1266	CA	PHE	165	33.133	35.301	44.953	1.00	10.86

FIG 5-20

ATOM	1267	C	PHE	165	32.751	36.445	44.071	1.00	15.53
ATOM	1268	O	PHE	165	31.686	37.020	44.251	1.00	17.16
ATOM	1269	CB	PHE	165	33.207	33.960	44.187	1.00	12.86
ATOM	1270	CG	PHE	165	31.862	33.486	43.622	1.00	14.35
ATOM	1271	CD1	PHE	165	31.510	33.749	42.293	1.00	14.61
ATOM	1272	CD2	PHE	165	30.978	32.757	44.413	1.00	13.56
ATOM	1273	CE1	PHE	165	30.300	33.297	41.759	1.00	22.67
ATOM	1274	CE2	PHE	165	29.774	32.282	43.893	1.00	15.76
ATOM	1275	CZ	PHE	165	29.426	32.572	42.573	1.00	16.20
ATOM	1276	N	LYS	166	33.641	36.799	43.132	1.00	10.79
ATOM	1277	CA	LYS	166	33.417	37.864	42.162	1.00	10.74
ATOM	1278	C	LYS	166	33.603	37.344	40.774	1.00	15.95
ATOM	1279	O	LYS	166	34.602	36.727	40.470	1.00	22.80
ATOM	1280	CB	LYS	166	34.387	39.055	42.249	1.00	16.61
ATOM	1281	CG	LYS	166	34.573	39.688	43.573	1.00	18.11
ATOM	1282	CD	LYS	166	35.540	40.875	43.454	1.00	32.56
ATOM	1283	CE	LYS	166	35.272	41.966	44.476	1.00	48.19
ATOM	1284	NZ	LYS	166	34.823	41.435	45.782	1.00	85.81
ATOM	1285	N	ILE	167	32.703	37.704	39.911	1.00	9.75
ATOM	1286	CA	ILE	167	32.768	37.340	38.558	1.00	9.35
ATOM	1287	C	ILE	167	33.203	38.542	37.823	1.00	14.36
ATOM	1288	O	ILE	167	32.811	39.640	38.170	1.00	16.22
ATOM	1289	CB	ILE	167	31.379	36.929	38.005	1.00	13.16
ATOM	1290	CG1	ILE	167	30.909	35.624	38.669	1.00	13.02
ATOM	1291	CG2	ILE	167	31.423	36.786	36.472	1.00	7.91
ATOM	1292	CD1	ILE	167	31.773	34.415	38.344	1.00	19.57
ATOM	1293	N	ARG	168	34.005	38.299	36.815	1.00	12.19
ATOM	1294	CA	ARG	168	34.500	39.308	35.945	1.00	15.07
ATOM	1295	C	ARG	168	33.948	39.122	34.528	1.00	16.64
ATOM	1296	O	ARG	168	34.278	38.156	33.836	1.00	17.70
ATOM	1297	CB	ARG	168	36.024	39.287	35.944	1.00	16.54
ATOM	1298	CG	ARG	168	36.580	39.632	37.321	1.00	25.54
ATOM	1299	CD	ARG	168	37.894	38.910	37.601	1.00	63.52
ATOM	1300	NE	ARG	168	38.380	38.191	36.416	1.00	73.52
ATOM	1301	CZ	ARG	168	38.764	36.926	36.416	1.00	67.92
ATOM	1302	NH1	ARG	168	38.795	36.192	37.527	1.00	57.44
ATOM	1303	NH2	ARG	168	39.192	36.375	35.271	1.00	59.15
ATOM	1304	N	HIS	169	33.090	40.064	34.098	1.00	14.88
ATOM	1305	CA	HIS	169	32.505	40.025	32.758	1.00	13.24
ATOM	1306	C	HIS	169	33.214	41.001	31.839	1.00	12.64
ATOM	1307	O	HIS	169	33.306	42.203	32.121	1.00	14.99
ATOM	1308	CB	HIS	169	30.970	40.374	32.760	1.00	10.46
ATOM	1309	CG	HIS	169	30.097	39.474	33.573	1.00	6.54
ATOM	1310	ND1	HIS	169	29.724	38.246	33.111	1.00	12.63
ATOM	1311	CD2	HIS	169	29.474	39.695	34.764	1.00	10.21
ATOM	1312	CE1	HIS	169	28.892	37.718	34.031	1.00	10.53
ATOM	1313	NE2	HIS	169	28.734	38.566	35.063	1.00	11.84
ATOM	1314	N	ASN	170	33.691	40.513	30.737	1.00	10.66
ATOM	1315	CA	ASN	170	34.349	41.368	29.812	1.00	15.87
ATOM	1316	C	ASN	170	33.356	42.224	29.067	1.00	25.06
ATOM	1317	O	ASN	170	32.386	41.701	28.537	1.00	16.60
ATOM	1318	CB	ASN	170	35.110	40.550	28.755	1.00	19.60
ATOM	1319	CG	ASN	170	36.245	39.717	29.312	1.00	18.70
ATOM	1320	OD1	ASN	170	36.702	38.752	28.684	1.00	48.29
ATOM	1321	ND2	ASN	170	36.695	40.073	30.480	1.00	19.13
ATOM	1322	N	ILE	171	33.662	43.527	28.947	1.00	18.75
ATOM	1323	CA	ILE	171	32.848	44.460	28.168	1.00	16.74
ATOM	1324	C	ILE	171	33.459	44.638	26.791	1.00	19.51
ATOM	1325	O	ILE	171	34.643	44.596	26.642	1.00	21.06
ATOM	1326	CB	ILE	171	32.713	45.804	28.842	1.00	20.46
ATOM	1327	CG1	ILE	171	32.089	45.617	30.193	1.00	24.79
ATOM	1328	CG2	ILE	171	31.852	46.727	27.997	1.00	19.03
ATOM	1329	CD1	ILE	171	32.630	46.599	31.229	1.00	41.65
ATOM	1330	N	GLU	172	32.632	44.818	25.804	1.00	16.54
ATOM	1331	CA	GLU	172	33.034	44.933	24.420	1.00	17.00
ATOM	1332	C	GLU	172	34.110	45.987	24.147	1.00	25.80
ATOM	1333	O	GLU	172	34.776	45.892	23.125	1.00	29.20

FIG 5-21

ATOM	1334	CB	GLU	172	31.813	45.165	23.509	1.00	22.46
ATOM	1335	CG	GLU	172	31.122	46.531	23.786	1.00	58.53
ATOM	1336	CD	GLU	172	29.871	46.783	22.933	1.00	100.00
ATOM	1337	OE1	GLU	172	29.415	45.970	22.156	1.00	100.00
ATOM	1338	OE2	GLU	172	29.370	47.982	23.149	1.00	100.00
ATOM	1339	N	ASP	173	34.277	46.934	25.034	1.00	24.41
ATOM	1340	CA	ASP	173	35.292	47.978	24.852	1.00	25.03
ATOM	1341	C	ASP	173	36.651	47.624	25.455	1.00	33.40
ATOM	1342	O	ASP	173	37.561	48.451	25.518	1.00	30.42
ATOM	1343	CB	ASP	173	34.822	49.319	25.401	1.00	23.30
ATOM	1344	CG	ASP	173	34.743	49.358	26.912	1.00	32.47
ATOM	1345	OD1	ASP	173	34.406	50.355	27.513	1.00	37.58
ATOM	1346	OD2	ASP	173	34.949	48.196	27.504	1.00	49.22
ATOM	1347	N	GLY	174	36.766	46.410	25.956	1.00	23.87
ATOM	1348	CA	GLY	174	38.019	45.994	26.537	1.00	21.30
ATOM	1349	C	GLY	174	38.012	46.090	28.044	1.00	19.99
ATOM	1350	O	GLY	174	38.927	45.585	28.709	1.00	20.45
ATOM	1351	N	SER	175	36.972	46.767	28.598	1.00	13.88
ATOM	1352	CA	SER	175	36.898	46.931	30.034	1.00	8.70
ATOM	1353	C	SER	175	36.296	45.728	30.765	1.00	17.30
ATOM	1354	O	SER	175	36.136	44.655	30.175	1.00	18.77
ATOM	1355	CB	SER	175	36.288	48.235	30.450	1.00	14.07
ATOM	1356	OG	SER	175	36.360	48.316	31.865	1.00	24.79
ATOM	1357	N	VAL	176	35.963	45.912	32.051	1.00	13.74
ATOM	1358	CA	VAL	176	35.415	44.826	32.864	1.00	16.46
ATOM	1359	C	VAL	176	34.191	45.204	33.703	1.00	22.46
ATOM	1360	O	VAL	176	34.159	46.254	34.334	1.00	21.31
ATOM	1361	CB	VAL	176	36.477	44.285	33.818	1.00	24.43
ATOM	1362	CG1	VAL	176	35.847	43.344	34.827	1.00	27.45
ATOM	1363	CG2	VAL	176	37.532	43.536	33.035	1.00	25.65
ATOM	1364	N	GLN	177	33.234	44.269	33.787	1.00	15.47
ATOM	1365	CA	GLN	177	32.048	44.430	34.647	1.00	15.40
ATOM	1366	C	GLN	177	32.102	43.457	35.813	1.00	10.60
ATOM	1367	O	GLN	177	32.027	42.243	35.634	1.00	13.65
ATOM	1368	CB	GLN	177	30.709	44.283	33.872	1.00	15.57
ATOM	1369	CG	GLN	177	29.468	44.294	34.828	1.00	19.13
ATOM	1370	CD	GLN	177	29.103	45.673	35.361	1.00	14.91
ATOM	1371	OE1	GLN	177	28.759	46.588	34.574	1.00	20.17
ATOM	1372	NE2	GLN	177	29.128	45.821	36.690	1.00	17.28
ATOM	1373	N	LEU	178	32.227	43.993	37.018	1.00	8.17
ATOM	1374	CA	LEU	178	32.313	43.180	38.181	1.00	16.66
ATOM	1375	C	LEU	178	30.954	42.786	38.712	1.00	20.93
ATOM	1376	O	LEU	178	30.033	43.608	38.753	1.00	14.66
ATOM	1377	CB	LEU	178	33.089	43.896	39.293	1.00	20.63
ATOM	1378	CG	LEU	178	34.286	43.110	39.815	1.00	39.28
ATOM	1379	CD1	LEU	178	33.831	42.087	40.852	1.00	45.14
ATOM	1380	CD2	LEU	178	35.018	42.426	38.648	1.00	39.52
ATOM	1381	N	ALA	179	30.869	41.550	39.171	1.00	16.72
ATOM	1382	CA	ALA	179	29.652	41.033	39.754	1.00	15.55
ATOM	1383	C	ALA	179	29.932	40.277	41.040	1.00	15.70
ATOM	1384	O	ALA	179	30.337	39.119	41.028	1.00	15.91
ATOM	1385	CB	ALA	179	28.853	40.197	38.731	1.00	14.08
ATOM	1386	N	ASP	180	29.694	40.946	42.155	1.00	8.88
ATOM	1387	CA	ASP	180	29.897	40.407	43.480	1.00	7.18
ATOM	1388	C	ASP	180	28.802	39.460	43.891	1.00	17.07
ATOM	1389	O	ASP	180	27.651	39.844	43.987	1.00	18.22
ATOM	1390	CB	ASP	180	29.934	41.509	44.509	1.00	13.06
ATOM	1391	CG	ASP	180	31.285	41.902	44.935	1.00	46.28
ATOM	1392	OD1	ASP	180	31.981	41.206	45.655	1.00	60.46
ATOM	1393	OD2	ASP	180	31.574	43.121	44.560	1.00	46.61
ATOM	1394	N	HIS	181	29.173	38.242	44.197	1.00	14.51
ATOM	1395	CA	HIS	181	28.213	37.223	44.575	1.00	10.49
ATOM	1396	C	HIS	181	28.218	36.897	46.049	1.00	14.28
ATOM	1397	O	HIS	181	29.255	36.530	46.607	1.00	17.40
ATOM	1398	CB	HIS	181	28.450	35.915	43.769	1.00	9.89
ATOM	1399	CG	HIS	181	28.077	35.972	42.328	1.00	10.38
ATOM	1400	ND1	HIS	181	28.606	36.926	41.455	1.00	12.24

FIG 5-22

ATOM	1401	CD2	HIS	181	27.279	35.146	41.606	1.00	10.42
ATOM	1402	CE1	HIS	181	28.093	36.678	40.269	1.00	9.97
ATOM	1403	NE2	HIS	181	27.314	35.594	40.316	1.00	9.38
ATOM	1404	N	TYR	182	27.029	36.897	46.668	1.00	10.40
ATOM	1405	CA	TYR	182	26.848	36.518	48.062	1.00	13.86
ATOM	1406	C	TYR	182	25.871	35.393	48.089	1.00	20.61
ATOM	1407	O	TYR	182	24.819	35.520	47.532	1.00	16.35
ATOM	1408	CB	TYR	182	26.359	37.664	48.934	1.00	21.12
ATOM	1409	CG	TYR	182	27.421	38.693	49.062	1.00	34.16
ATOM	1410	CD1	TYR	182	27.521	39.715	48.120	1.00	46.06
ATOM	1411	CD2	TYR	182	28.389	38.616	50.064	1.00	38.56
ATOM	1412	CE1	TYR	182	28.532	40.674	48.197	1.00	57.53
ATOM	1413	CE2	TYR	182	29.418	39.559	50.147	1.00	40.76
ATOM	1414	CZ	TYR	182	29.480	40.594	49.216	1.00	54.61
ATOM	1415	OH	TYR	182	30.461	41.534	49.308	1.00	61.92
ATOM	1416	N	GLN	183	26.246	34.277	48.686	1.00	17.63
ATOM	1417	CA	GLN	183	25.410	33.104	48.583	1.00	16.37
ATOM	1418	C	GLN	183	25.289	32.311	49.863	1.00	21.39
ATOM	1419	O	GLN	183	26.260	32.174	50.623	1.00	19.86
ATOM	1420	CB	GLN	183	25.984	32.219	47.422	1.00	13.33
ATOM	1421	CG	GLN	183	25.651	30.688	47.457	1.00	17.38
ATOM	1422	CD	GLN	183	26.411	29.884	46.389	1.00	17.27
ATOM	1423	OE1	GLN	183	26.975	30.454	45.456	1.00	13.80
ATOM	1424	NE2	GLN	183	26.361	28.553	46.473	1.00	13.94
ATOM	1425	N	GLN	184	24.080	31.739	50.055	1.00	19.74
ATOM	1426	CA	GLN	184	23.760	30.829	51.168	1.00	16.55
ATOM	1427	C	GLN	184	23.033	29.582	50.658	1.00	13.60
ATOM	1428	O	GLN	184	22.219	29.640	49.747	1.00	18.01
ATOM	1429	CB	GLN	184	22.949	31.444	52.330	1.00	20.11
ATOM	1430	CG	GLN	184	23.364	32.855	52.768	1.00	74.84
ATOM	1431	CD	GLN	184	22.312	33.517	53.657	1.00	100.00
ATOM	1432	OE1	GLN	184	21.159	33.054	53.752	1.00	97.99
ATOM	1433	NE2	GLN	184	22.689	34.625	54.286	1.00	100.00
ATOM	1434	N	ASN	185	23.418	23.446	51.207	1.00	14.76
ATOM	1435	CA	ASN	185	22.831	27.155	50.887	1.00	13.86
ATOM	1436	C	ASN	185	22.421	26.463	52.166	1.00	16.06
ATOM	1437	O	ASN	185	23.176	26.402	53.172	1.00	17.39
ATOM	1438	CB	ASN	185	23.761	26.212	50.119	1.00	15.20
ATOM	1439	CG	ASN	185	24.110	26.696	48.748	1.00	12.75
ATOM	1440	OD1	ASN	185	24.704	27.758	48.592	1.00	22.56
ATOM	1441	ND2	ASN	185	23.830	25.868	47.763	1.00	17.70
ATOM	1442	N	THR	186	21.227	25.941	52.139	1.00	18.01
ATOM	1443	CA	THR	186	20.707	25.227	53.288	1.00	17.40
ATOM	1444	C	THR	186	19.976	24.010	52.824	1.00	23.63
ATOM	1445	O	THR	186	19.389	23.991	51.730	1.00	24.57
ATOM	1446	CB	THR	186	19.856	26.100	54.206	1.00	28.82
ATOM	1447	OG1	THR	186	18.874	26.752	53.446	1.00	35.65
ATOM	1448	CG2	THR	186	20.753	27.121	54.903	1.00	28.86
ATOM	1449	N	PRO	187	20.101	22.951	53.620	1.00	22.40
ATOM	1450	CA	PRO	187	19.504	21.683	53.269	1.00	20.28
ATOM	1451	C	PRO	187	17.988	21.757	53.288	1.00	22.41
ATOM	1452	O	PRO	187	17.390	22.518	54.071	1.00	25.07
ATOM	1453	CB	PRO	187	19.977	20.682	54.337	1.00	19.79
ATOM	1454	CG	PRO	187	20.840	21.449	55.338	1.00	26.98
ATOM	1455	CD	PRO	187	20.786	22.918	54.949	1.00	22.04
ATOM	1456	N	ILE	188	17.382	20.957	52.453	1.00	18.77
ATOM	1457	CA	ILE	188	15.907	20.855	52.407	1.00	20.12
ATOM	1458	C	ILE	188	15.470	19.766	53.389	1.00	31.58
ATOM	1459	O	ILE	188	14.596	19.966	54.202	1.00	38.58
ATOM	1460	CB	ILE	188	15.385	20.574	50.991	1.00	21.52
ATOM	1461	CG1	ILE	188	15.555	21.775	50.102	1.00	16.10
ATOM	1462	CG2	ILE	188	13.916	20.141	50.981	1.00	28.85
ATOM	1463	CD1	ILE	188	15.139	21.471	48.660	1.00	15.31
ATOM	1464	N	GLY	189	16.142	18.618	53.352	1.00	32.39
ATOM	1465	CA	GLY	189	15.833	17.531	54.283	1.00	32.94
ATOM	1466	C	GLY	189	16.339	17.817	55.702	1.00	40.20
ATOM	1467	O	GLY	189	17.016	18.810	55.967	1.00	35.57

FIG 5-23

ATOM	1468	N	ASP	190	16.003	16.928	56.617	1.00	49.41
ATOM	1469	CA	ASP	190	16.392	17.047	58.021	1.00	55.01
ATOM	1470	C	ASP	190	17.556	16.115	58.338	1.00	56.16
ATOM	1471	O	ASP	190	18.083	16.100	59.463	1.00	58.30
ATOM	1472	CB	ASP	190	15.195	16.734	58.955	1.00	63.89
ATOM	1473	CG	ASP	190	14.592	15.365	58.686	1.00	99.67
ATOM	1474	OD1	ASP	190	14.599	14.466	59.514	1.00	100.00
ATOM	1475	OD2	ASP	190	14.088	15.240	57.470	1.00	100.00
ATOM	1476	N	GLY	191	17.921	15.312	57.323	1.00	47.20
ATOM	1477	CA	GLY	191	19.015	14.347	57.419	1.00	44.96
ATOM	1478	C	GLY	191	20.359	15.044	57.587	1.00	34.43
ATOM	1479	O	GLY	191	20.452	16.266	57.438	1.00	29.96
ATOM	1480	N	PRO	192	21.402	14.264	57.905	1.00	27.26
ATOM	1481	CA	PRO	192	22.737	14.834	58.100	1.00	24.01
ATOM	1482	C	PRO	192	23.444	15.274	56.787	1.00	20.55
ATOM	1483	O	PRO	192	23.323	14.648	55.740	1.00	23.84
ATOM	1484	CB	PRO	192	23.583	13.764	58.825	1.00	21.00
ATOM	1485	CG	PRO	192	22.739	12.501	58.915	1.00	27.49
ATOM	1486	CD	PRO	192	21.330	12.863	58.448	1.00	27.26
ATOM	1487	N	VAL	193	24.193	16.363	56.892	1.00	17.87
ATOM	1488	CA	VAL	193	24.964	16.902	55.792	1.00	19.51
ATOM	1489	C	VAL	193	26.380	17.108	56.249	1.00	22.37
ATOM	1490	O	VAL	193	26.663	17.189	57.443	1.00	23.84
ATOM	1491	CB	VAL	193	24.449	18.245	55.256	1.00	25.24
ATOM	1492	CG1	VAL	193	23.059	18.118	54.632	1.00	21.90
ATOM	1493	CG2	VAL	193	24.497	19.322	56.346	1.00	24.81
ATOM	1494	N	LEU	194	27.253	17.241	55.277	1.00	19.04
ATOM	1495	CA	LEU	194	28.654	17.438	55.516	1.00	20.29
ATOM	1496	C	LEU	194	29.006	18.930	55.571	1.00	18.71
ATOM	1497	O	LEU	194	28.907	19.615	54.591	1.00	20.13
ATOM	1498	CB	LEU	194	29.412	16.806	54.327	1.00	22.92
ATOM	1499	CG	LEU	194	29.994	15.423	54.542	1.00	30.60
ATOM	1500	CD1	LEU	194	29.227	14.642	55.595	1.00	35.19
ATOM	1501	CD2	LEU	194	30.048	14.672	53.211	1.00	25.61
ATOM	1502	N	LEU	195	29.453	19.430	56.713	1.00	17.39
ATOM	1503	CA	LEU	195	29.881	20.808	56.785	1.00	18.83
ATOM	1504	C	LEU	195	31.389	20.837	56.579	1.00	28.32
ATOM	1505	O	LEU	195	32.161	20.152	57.281	1.00	21.98
ATOM	1506	CB	LEU	195	29.489	21.525	58.072	1.00	22.20
ATOM	1507	CG	LEU	195	28.055	21.349	58.444	1.00	26.40
ATOM	1508	CD1	LEU	195	27.937	21.508	59.941	1.00	31.99
ATOM	1509	CD2	LEU	195	27.225	22.395	57.726	1.00	26.90
ATOM	1510	N	PRO	196	31.789	21.610	55.597	1.00	21.58
ATOM	1511	CA	PRO	196	33.177	21.666	55.154	1.00	22.17
ATOM	1512	C	PRO	196	34.080	22.623	55.892	1.00	29.56
ATOM	1513	O	PRO	196	33.635	23.588	56.490	1.00	29.04
ATOM	1514	CB	PRO	196	33.054	22.265	53.752	1.00	22.77
ATOM	1515	CG	PRO	196	31.761	23.104	53.735	1.00	18.99
ATOM	1516	CD	PRO	196	30.910	22.567	54.861	1.00	16.42
ATOM	1517	N	ASP	197	35.379	22.410	55.716	1.00	22.95
ATOM	1518	CA	ASP	197	36.364	23.370	56.134	1.00	19.71
ATOM	1519	C	ASP	197	36.556	24.295	54.931	1.00	24.74
ATOM	1520	O	ASP	197	36.251	23.913	53.800	1.00	24.88
ATOM	1521	CB	ASP	197	37.711	22.730	56.446	1.00	22.28
ATOM	1522	CG	ASP	197	37.690	21.913	57.687	1.00	43.93
ATOM	1523	OD1	ASP	197	36.912	22.117	58.608	1.00	53.47
ATOM	1524	OD2	ASP	197	38.634	21.006	57.694	1.00	31.58
ATOM	1525	N	ASN	198	37.062	25.501	55.168	1.00	19.74
ATOM	1526	CA	ASN	198	37.254	26.470	54.118	1.00	15.38
ATOM	1527	C	ASN	198	37.974	25.889	52.971	1.00	19.61
ATOM	1528	O	ASN	198	38.958	25.236	53.134	1.00	22.69
ATOM	1529	CB	ASN	198	38.013	27.704	54.614	1.00	24.48
ATOM	1530	CG	ASN	198	37.236	28.504	55.632	1.00	52.21
ATOM	1531	OD1	ASN	198	36.107	28.174	55.961	1.00	34.54
ATOM	1532	ND2	ASN	198	37.854	29.556	56.150	1.00	55.11
ATOM	1533	N	HIS	199	37.462	26.125	51.801	1.00	16.30
ATOM	1534	CA	HIS	199	38.071	25.627	50.616	1.00	15.30

FIG 5-24

ATOM	1535	C	HIS	199	37.496	26.357	49.450	1.00	14.85
ATOM	1536	O	HIS	199	36.757	27.295	49.643	1.00	16.45
ATOM	1537	CB	HIS	199	37.988	24.103	50.471	1.00	16.53
ATOM	1538	CG	HIS	199	36.597	23.628	50.218	1.00	16.65
ATOM	1539	ND1	HIS	199	35.695	23.491	51.244	1.00	17.85
ATOM	1540	CD2	HIS	199	35.987	23.282	49.048	1.00	18.67
ATOM	1541	CE1	HIS	199	34.561	23.052	50.688	1.00	19.45
ATOM	1542	NE2	HIS	199	34.716	22.905	49.364	1.00	18.74
ATOM	1543	N	TYR	200	37.879	25.998	48.247	1.00	12.56
ATOM	1544	CA	TYR	200	37.334	26.689	47.100	1.00	14.01
ATOM	1545	C	TYR	200	37.207	25.824	45.870	1.00	15.57
ATOM	1546	O	TYR	200	37.793	24.751	45.768	1.00	20.20
ATOM	1547	CB	TYR	200	38.030	28.011	46.779	1.00	19.79
ATOM	1548	CG	TYR	200	39.382	27.745	46.202	1.00	22.25
ATOM	1549	CD1	TYR	200	39.543	27.526	44.835	1.00	22.53
ATOM	1550	CD2	TYR	200	40.473	27.605	47.057	1.00	25.73
ATOM	1551	CE1	TYR	200	40.800	27.222	44.317	1.00	35.51
ATOM	1552	CE2	TYR	200	41.739	27.314	46.559	1.00	29.34
ATOM	1553	CZ	TYR	200	41.896	27.132	45.186	1.00	54.14
ATOM	1554	OH	TYR	200	43.153	26.820	44.703	1.00	62.66
ATOM	1555	N	LEU	201	36.393	26.309	44.946	1.00	15.07
ATOM	1556	CA	LEU	201	36.147	25.680	43.678	1.00	11.01
ATOM	1557	C	LEU	201	36.753	26.532	42.593	1.00	17.30
ATOM	1558	O	LEU	201	36.619	27.753	42.610	1.00	20.19
ATOM	1559	CB	LEU	201	34.628	25.518	43.354	1.00	10.09
ATOM	1560	CG	LEU	201	33.749	25.027	44.480	1.00	13.41
ATOM	1561	CD1	LEU	201	32.293	24.938	43.954	1.00	17.11
ATOM	1562	CD2	LEU	201	34.196	23.635	44.927	1.00	23.03
ATOM	1563	N	SER	202	37.407	25.868	41.651	1.00	10.75
ATOM	1564	CA	SER	202	38.047	26.490	40.528	1.00	8.51
ATOM	1565	C	SER	202	37.222	26.189	39.294	1.00	11.56
ATOM	1566	O	SER	202	36.919	25.038	38.996	1.00	14.58
ATOM	1567	CB	SER	202	39.485	25.987	40.442	1.00	15.68
ATOM	1568	OG	SER	202	40.067	26.353	39.228	1.00	36.44
ATOM	1569	N	THR	203	36.798	27.241	38.601	1.00	12.36
ATOM	1570	CA	THR	203	35.879	27.067	37.499	1.00	15.60
ATOM	1571	C	THR	203	36.417	27.521	36.195	1.00	20.19
ATOM	1572	O	THR	203	37.192	28.472	36.114	1.00	18.29
ATOM	1573	CB	THR	203	34.565	27.892	37.757	1.00	20.51
ATOM	1574	OG1	THR	203	34.911	29.260	37.780	1.00	20.39
ATOM	1575	CG2	THR	203	33.935	27.557	39.093	1.00	6.80
ATOM	1576	N	GLN	204	35.913	26.883	35.164	1.00	10.30
ATOM	1577	CA	GLN	204	36.173	27.271	33.807	1.00	14.85
ATOM	1578	C	GLN	204	34.956	26.980	32.921	1.00	23.14
ATOM	1579	O	GLN	204	34.334	25.932	33.056	1.00	21.66
ATOM	1580	CB	GLN	204	37.475	26.696	33.237	1.00	20.33
ATOM	1581	CG	GLN	204	37.271	25.371	32.518	1.00	40.16
ATOM	1582	CD	GLN	204	38.588	24.722	32.193	1.00	59.76
ATOM	1583	OE1	GLN	204	39.011	24.716	31.035	1.00	41.80
ATOM	1584	NE2	GLN	204	39.276	24.241	33.235	1.00	34.80
ATOM	1585	N	SER	205	34.619	27.913	32.021	1.00	15.83
ATOM	1586	CA	SER	205	33.447	27.762	31.172	1.00	14.60
ATOM	1587	C	SER	205	33.654	28.307	29.783	1.00	20.21
ATOM	1588	O	SER	205	34.282	29.337	29.581	1.00	17.82
ATOM	1589	CB	SER	205	32.197	28.445	31.758	1.00	11.88
ATOM	1590	OG	SER	205	32.121	28.406	33.177	1.00	15.45
ATOM	1591	N	ALA	206	33.065	27.630	28.827	1.00	13.00
ATOM	1592	CA	ALA	206	33.079	28.029	27.426	1.00	9.99
ATOM	1593	C	ALA	206	31.623	28.192	26.924	1.00	21.23
ATOM	1594	O	ALA	206	30.809	27.306	27.139	1.00	14.10
ATOM	1595	CB	ALA	206	33.751	26.936	26.596	1.00	13.45
ATOM	1596	N	LEU	207	31.335	29.320	26.263	1.00	16.09
ATOM	1597	CA	LEU	207	30.036	29.617	25.706	1.00	12.07
ATOM	1598	C	LEU	207	30.070	29.445	24.235	1.00	19.76
ATOM	1599	O	LEU	207	31.014	29.840	23.576	1.00	20.82
ATOM	1600	CB	LEU	207	29.580	31.057	26.004	1.00	8.24
ATOM	1601	CG	LEU	207	29.744	31.493	27.457	1.00	16.35

FIG 5-25

```
ATOM   1602  CD1 LEU   207      28.955  32.790  27.707  1.00 13.78
ATOM   1603  CD2 LEU   207      29.268  30.406  28.400  1.00 18.79
ATOM   1604  N   SER   208      29.011  28.863  23.698  1.00 15.35
ATOM   1605  CA  SER   208      28.914  28.692  22.270  1.00 13.74
ATOM   1606  C   SER   208      27.449  28.852  21.794  1.00 20.16
ATOM   1607  O   SER   208      26.548  29.085  22.594  1.00 15.81
ATOM   1608  CB  SER   208      29.495  27.367  21.822  1.00 17.82
ATOM   1609  OG  SER   208      28.769  26.311  22.431  1.00 31.45
ATOM   1610  N   LYS   209      27.242  28.738  20.485  1.00 16.50
ATOM   1611  CA  LYS   209      25.907  28.828  19.906  1.00 18.02
ATOM   1612  C   LYS   209      25.637  27.610  19.031  1.00 29.99
ATOM   1613  O   LYS   209      26.578  27.004  18.502  1.00 32.55
ATOM   1614  CB  LYS   209      25.783  30.100  19.082  1.00 20.96
ATOM   1615  CG  LYS   209      24.746  31.055  19.606  1.00 34.50
ATOM   1616  CD  LYS   209      25.262  31.964  20.666  1.00 22.72
ATOM   1617  CE  LYS   209      24.370  33.159  20.896  1.00 18.96
ATOM   1618  NZ  LYS   209      23.565  33.067  22.116  1.00 27.39
ATOM   1619  N   ASP   210      24.347  27.241  18.912  1.00 27.01
ATOM   1620  CA  ASP   210      23.890  26.159  18.038  1.00 24.62
ATOM   1621  C   ASP   210      23.465  26.793  16.705  1.00 26.77
ATOM   1622  O   ASP   210      22.468  27.514  16.605  1.00 23.00
ATOM   1623  CB  ASP   210      22.744  25.361  18.691  1.00 24.43
ATOM   1624  CG  ASP   210      22.197  24.249  17.839  1.00 35.55
ATOM   1625  OD1 ASP   210      22.333  24.185  16.631  1.00 36.53
ATOM   1626  OD2 ASP   210      21.499  23.400  18.535  1.00 45.51
ATOM   1627  N   PRO   211      24.306  26.618  15.708  1.00 30.25
ATOM   1628  CA  PRO   211      24.120  27.224  14.397  1.00 30.30
ATOM   1629  C   PRO   211      22.733  26.982  13.770  1.00 39.72
ATOM   1630  O   PRO   211      22.253  27.782  12.959  1.00 37.65
ATOM   1631  CB  PRO   211      25.197  26.620  13.500  1.00 29.99
ATOM   1632  CG  PRO   211      25.782  25.418  14.255  1.00 38.59
ATOM   1633  CD  PRO   211      25.158  25.405  15.647  1.00 35.05
ATOM   1634  N   ASN   212      22.102  25.868  14.140  1.00 39.64
ATOM   1635  CA  ASN   212      20.808  25.515  13.592  1.00 39.60
ATOM   1636  C   ASN   212      19.642  25.894  14.497  1.00 41.92
ATOM   1637  O   ASN   212      18.485  25.518  14.263  1.00 42.30
ATOM   1638  CB  ASN   212      20.738  24.028  13.236  1.00 48.64
ATOM   1639  CG  ASN   212      21.883  23.678  12.230  1.00 53.61
ATOM   1640  N   GLU   213      19.947  26.675  15.520  1.00 27.84
ATOM   1641  CA  GLU   213      18.953  27.080  16.478  1.00 20.43
ATOM   1642  C   GLU   213      18.485  28.527  16.241  1.00 29.95
ATOM   1643  O   GLU   213      19.247  29.475  16.324  1.00 32.77
ATOM   1644  CB  GLU   213      19.535  26.878  17.894  1.00 16.45
ATOM   1645  CG  GLU   213      18.594  27.326  18.995  1.00 18.29
ATOM   1646  CD  GLU   213      17.229  26.703  18.853  1.00 38.01
ATOM   1647  OE1 GLU   213      16.238  27.334  18.508  1.00 25.07
ATOM   1648  OE2 GLU   213      17.223  25.423  19.122  1.00 19.17
ATOM   1649  N   LYS   214      17.223  28.713  15.963  1.00 22.99
ATOM   1650  CA  LYS   214      16.721  30.081  15.726  1.00 22.84
ATOM   1651  C   LYS   214      16.252  30.778  16.982  1.00 21.50
ATOM   1652  O   LYS   214      16.130  32.016  17.032  1.00 28.15
ATOM   1653  CB  LYS   214      15.653  30.197  14.606  1.00 27.58
ATOM   1654  CG  LYS   214      16.153  29.816  13.209  1.00 32.71
ATOM   1655  CD  LYS   214      16.752  30.979  12.431  1.00 58.31
ATOM   1656  N   ARG   215      15.947  30.028  13.014  1.00 14.52
ATOM   1657  CA  ARG   215      15.518  30.726  19.209  1.00 15.58
ATOM   1658  C   ARG   215      16.719  31.382  19.892  1.00 21.87
ATOM   1659  O   ARG   215      17.848  31.075  19.572  1.00 26.69
ATOM   1660  CB  ARG   215      14.808  29.804  20.159  1.00 18.82
ATOM   1661  CG  ARG   215      13.660  29.067  19.475  1.00 23.30
ATOM   1662  CD  ARG   215      13.220  27.806  20.205  1.00 15.45
ATOM   1663  NE  ARG   215      14.107  26.668  19.929  1.00 28.08
ATOM   1664  CZ  ARG   215      14.022  25.473  20.543  1.00 21.38
ATOM   1665  NH1 ARG   215      13.074  25.215  21.455  1.00 23.92
ATOM   1666  NH2 ARG   215      14.893  24.514  20.225  1.00 20.46
ATOM   1667  N   ASP   216      16.466  32.275  20.830  1.00 16.72
ATOM   1668  CA  ASP   216      17.556  32.895  21.617  1.00 19.06
```

FIG 5-26

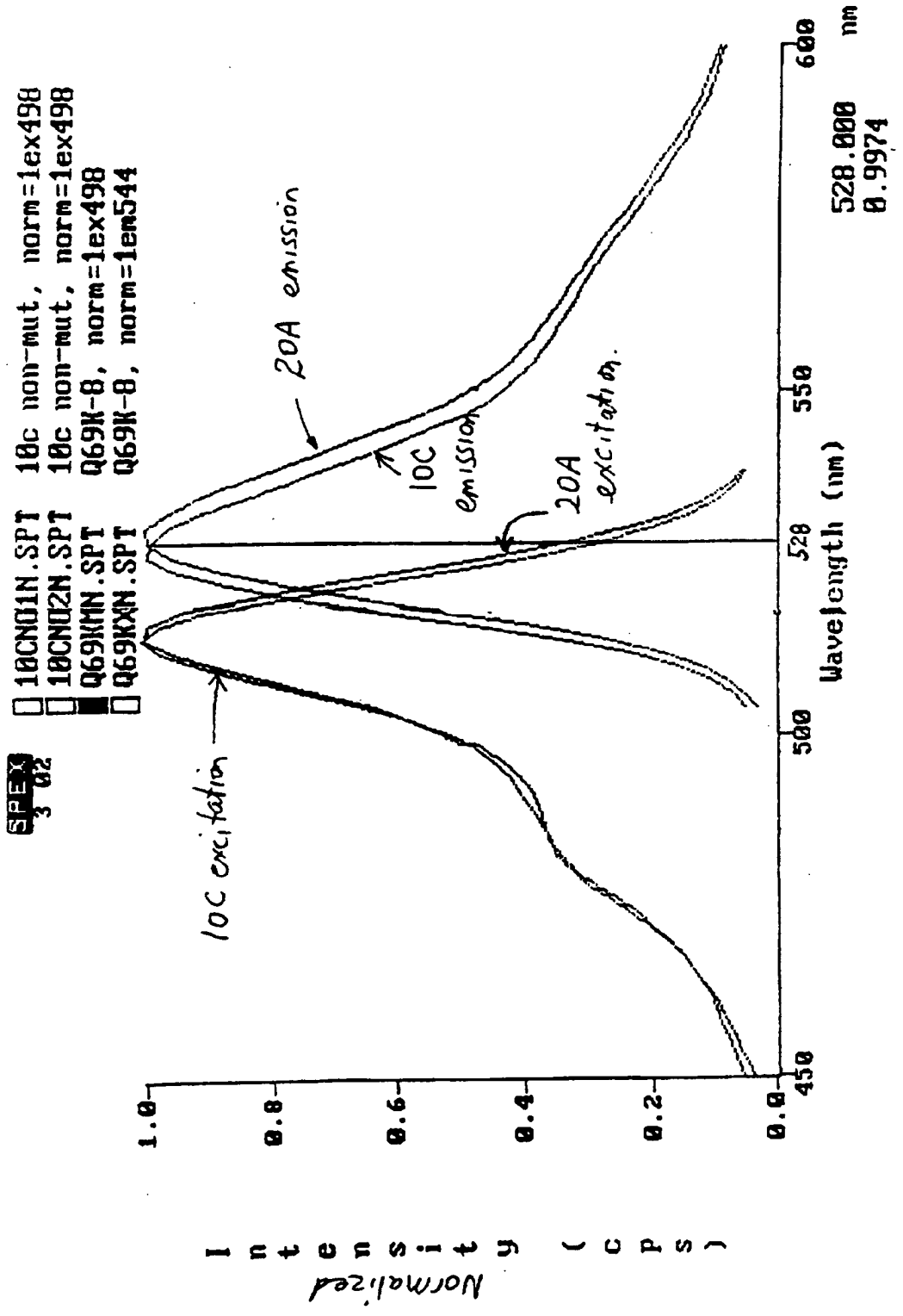
```
ATOM   1669  C   ASP  216      18.047  31.817  22.607  1.00 20.02
ATOM   1670  O   ASP  216      17.261  31.214  23.350  1.00 18.45
ATOM   1671  CB  ASP  216      17.066  34.169  22.383  1.00 21.33
ATOM   1672  CG  ASP  216      18.138  35.140  22.893  1.00 20.97
ATOM   1673  OD1 ASP  216      17.869  36.079  23.620  1.00 28.46
ATOM   1674  OD2 ASP  216      19.342  34.900  22.441  1.00 20.37
ATOM   1675  N   HIS  217      19.332  31.537  22.589  1.00 13.18
ATOM   1676  CA  HIS  217      19.813  30.482  23.433  1.00 11.21
ATOM   1677  C   HIS  217      21.313  30.614  23.723  1.00 21.35
ATOM   1678  O   HIS  217      22.014  31.471  23.163  1.00 15.03
ATOM   1679  CB  HIS  217      19.587  29.168  22.690  1.00 13.03
ATOM   1680  CG  HIS  217      20.525  29.025  21.542  1.00 15.49
ATOM   1681  ND1 HIS  217      20.463  29.871  20.449  1.00 17.88
ATOM   1682  CD2 HIS  217      21.589  28.172  21.361  1.00 17.51
ATOM   1683  CE1 HIS  217      21.457  29.524  19.635  1.00 17.94
ATOM   1684  NE2 HIS  217      22.152  28.501  20.151  1.00 17.59
ATOM   1685  N   MSE  218      21.794  29.725  24.576  1.00 11.26
ATOM   1686  CA  MSE  218      23.186  29.642  24.887  1.00 11.49
ATOM   1687  C   MSE  218      23.560  28.198  25.094  1.00 24.15
ATOM   1688  O   MSE  218      22.822  27.446  25.751  1.00 20.70
ATOM   1689  CB  MSE  218      23.539  30.421  26.172  1.00 12.84
ATOM   1690  CG  MSE  218      24.809  30.004  26.907  1.00 12.59
ATOM   1691  SE  MSE  218      25.267  31.128  28.434  1.00 29.94
ATOM   1692  CE  MSE  218      24.039  30.502  29.781  1.00 13.54
ATOM   1693  N   VAL  219      24.727  27.824  24.558  1.00 15.62
ATOM   1694  CA  VAL  219      25.309  26.518  24.782  1.00 10.58
ATOM   1695  C   VAL  219      26.473  26.689  25.753  1.00 16.54
ATOM   1696  O   VAL  219      27.280  27.604  25.585  1.00 15.54
ATOM   1697  CB  VAL  219      25.774  25.883  23.498  1.00 15.08
ATOM   1698  CG1 VAL  219      26.330  24.495  23.824  1.00 14.34
ATOM   1699  CG2 VAL  219      24.599  25.766  22.512  1.00 15.78
ATOM   1700  N   LEU  220      26.523  25.836  26.783  1.00 10.95
ATOM   1701  CA  LEU  220      27.490  25.939  27.850  1.00 11.01
ATOM   1702  C   LEU  220      28.206  24.643  28.184  1.00 21.26
ATOM   1703  O   LEU  220      27.592  23.577  28.324  1.00 15.94
ATOM   1704  CB  LEU  220      26.807  26.545  29.100  1.00 13.75
ATOM   1705  CG  LEU  220      27.624  26.578  30.402  1.00 21.10
ATOM   1706  CD1 LEU  220      28.433  27.875  30.483  1.00 23.53
ATOM   1707  CD2 LEU  220      26.663  26.556  31.586  1.00 22.04
ATOM   1708  N   LEU  221      29.570  24.758  28.273  1.00 19.04
ATOM   1709  CA  LEU  221      30.498  23.666  28.697  1.00 13.22
ATOM   1710  C   LEU  221      31.309  24.178  29.887  1.00 10.73
ATOM   1711  O   LEU  221      31.846  25.267  29.857  1.00 12.98
ATOM   1712  CB  LEU  221      31.382  23.102  27.549  1.00 13.74
ATOM   1713  CG  LEU  221      32.580  22.257  28.045  1.00 18.64
ATOM   1714  CD1 LEU  221      32.149  20.868  28.496  1.00 17.38
ATOM   1715  CD2 LEU  221      33.571  22.109  26.911  1.00 26.97
ATOM   1716  N   GLU  222      31.316  23.446  30.963  1.00  9.31
ATOM   1717  CA  GLU  222      31.936  23.929  32.144  1.00  9.97
ATOM   1718  C   GLU  222      32.548  22.803  32.951  1.00 12.94
ATOM   1719  O   GLU  222      32.072  21.662  32.966  1.00 13.38
ATOM   1720  CB  GLU  222      30.836  24.762  32.896  1.00 12.14
ATOM   1721  CG  GLU  222      31.092  25.119  34.364  1.00 13.88
ATOM   1722  CD  GLU  222      29.895  25.891  34.934  1.00 13.57
ATOM   1723  OE1 GLU  222      29.128  26.477  34.240  1.00 19.47
ATOM   1724  OE2 GLU  222      29.752  25.789  36.207  1.00 18.51
ATOM   1725  N   PHE  223      33.687  23.123  33.542  1.00 15.86
ATOM   1726  CA  PHE  223      34.476  22.227  34.373  1.00  9.34
ATOM   1727  C   PHE  223      34.711  22.864  35.722  1.00 11.08
ATOM   1728  O   PHE  223      35.028  24.055  35.828  1.00 19.86
ATOM   1729  CB  PHE  223      35.847  21.919  33.684  1.00  8.30
ATOM   1730  CG  PHE  223      35.703  21.134  32.431  1.00 10.50
ATOM   1731  CD1 PHE  223      35.570  19.747  32.469  1.00 13.56
ATOM   1732  CD2 PHE  223      35.750  21.750  31.184  1.00 11.32
ATOM   1733  CE1 PHE  223      35.481  19.010  31.287  1.00 12.58
ATOM   1734  CE2 PHE  223      35.667  21.032  29.995  1.00 12.17
ATOM   1735  CZ  PHE  223      35.521  19.648  30.050  1.00 10.87
```

FIG 5-27

ATOM	1736	N	VAL	224	34.542	22.081	36.765	1.00	9.28
ATOM	1737	CA	VAL	224	34.708	22.587	38.080	1.00	11.18
ATOM	1738	C	VAL	224	35.324	21.553	39.010	1.00	17.52
ATOM	1739	O	VAL	224	34.848	20.418	39.137	1.00	13.17
ATOM	1740	CB	VAL	224	33.370	23.078	38.662	1.00	16.61
ATOM	1741	CG1	VAL	224	33.622	23.736	40.022	1.00	13.90
ATOM	1742	CG2	VAL	224	32.674	24.048	37.697	1.00	13.85
ATOM	1743	N	THR	225	36.380	21.965	39.676	1.00	11.71
ATOM	1744	CA	THR	225	37.026	21.099	40.617	1.00	11.61
ATOM	1745	C	THR	225	37.366	21.798	41.927	1.00	14.76
ATOM	1746	O	THR	225	37.702	23.002	41.962	1.00	16.64
ATOM	1747	CB	THR	225	38.162	20.279	40.014	1.00	20.38
ATOM	1748	OG1	THR	225	39.288	20.337	40.822	1.00	30.44
ATOM	1749	CG2	THR	225	38.468	20.722	38.631	1.00	10.89
ATOM	1750	N	ALA	226	37.222	21.065	43.011	1.00	7.89
ATOM	1751	CA	ALA	226	37.478	21.595	44.352	1.00	11.63
ATOM	1752	C	ALA	226	38.969	21.558	44.677	1.00	16.61
ATOM	1753	O	ALA	226	39.687	20.699	44.199	1.00	15.60
ATOM	1754	CB	ALA	226	36.695	20.847	45.444	1.00	12.17
ATOM	1755	N	ALA	227	39.395	22.490	45.479	1.00	13.95
ATOM	1756	CA	ALA	227	40.789	22.550	45.871	1.00	19.64
ATOM	1757	C	ALA	227	40.987	23.299	47.170	1.00	26.33
ATOM	1758	O	ALA	227	40.042	23.715	47.840	1.00	25.39
ATOM	1759	CB	ALA	227	41.557	23.246	44.760	1.00	18.42
ATOM	1760	N	GLY	228	42.245	23.476	47.523	1.00	23.28
ATOM	1761	CA	GLY	228	42.616	24.292	48.658	1.00	21.61
ATOM	1762	C	GLY	228	42.805	23.562	49.939	1.00	32.93
ATOM	1763	O	GLY	228	42.948	24.201	51.009	1.00	32.53
ATOM	1764	N	ILE	229	42.803	22.231	49.842	1.00	33.59
ATOM	1765	CA	ILE	229	43.006	21.375	50.998	1.00	31.81
ATOM	1766	C	ILE	229	44.016	20.291	50.633	1.00	28.78
ATOM	1767	O	ILE	229	45.090	20.176	51.246	1.00	96.02
ATOM	1768	CB	ILE	229	41.691	20.772	51.519	1.00	35.70
ATOM	1769	CG1	ILE	229	40.890	21.807	52.325	1.00	30.66
ATOM	1770	CG2	ILE	229	41.990	19.549	52.392	1.00	33.37
ATOM	1771	CD1	ILE	229	39.386	21.715	52.092	1.00	38.74
ATOM	1772	O	HOH	301	27.530	12.735	38.010	1.00	15.09
ATOM	1773	O	HOH	302	23.919	34.589	37.331	1.00	10.29
ATOM	1774	O	HOH	303	27.229	34.816	35.487	1.00	11.12
ATOM	1775	O	HOH	304	29.914	18.943	44.692	1.00	16.10
ATOM	1776	O	HOH	305	30.956	21.886	49.900	1.00	21.47
ATOM	1777	O	HOH	306	20.072	31.196	43.592	1.00	16.85
ATOM	1778	O	HOH	307	26.660	48.630	33.797	1.00	24.67
ATOM	1779	O	HOH	308	22.329	33.239	41.399	1.00	14.11
ATOM	1780	O	HOH	309	22.465	48.025	32.810	1.00	18.51
ATOM	1781	O	HOH	310	31.012	39.126	29.118	1.00	16.01
ATOM	1782	O	HOH	311	33.067	35.809	33.010	1.00	19.92
ATOM	1783	O	HOH	312	31.130	37.076	30.841	1.00	12.58
ATOM	1784	O	HOH	313	40.304	30.058	38.616	1.00	56.07
ATOM	1785	O	HOH	314	34.166	26.379	57.222	1.00	22.58
ATOM	1786	O	HOH	315	36.215	35.320	43.598	1.00	22.30
ATOM	1787	O	HOH	316	33.866	29.786	34.671	1.00	12.21
ATOM	1865	O	HOH	317	42.341	20.166	43.534	1.00	26.67
ATOM	1788	O	HOH	318	10.270	28.684	30.403	1.00	43.66
ATOM	1789	O	HOH	319	28.448	16.822	30.655	1.00	25.44
ATOM	1790	O	HOH	320	30.612	20.922	37.231	1.00	21.57
ATOM	1791	O	HOH	321	11.639	37.421	26.801	1.00	34.12
ATOM	1792	O	HOH	322	27.030	37.308	36.869	1.00	13.10
ATOM	1793	O	HOH	323	33.119	14.524	43.070	1.00	30.93
ATOM	1794	O	HOH	324	37.973	14.036	53.352	1.00	38.39
ATOM	1795	O	HOH	325	32.015	49.100	37.028	1.00	59.37
ATOM	1796	O	HOH	326	11.959	12.020	43.429	1.00	29.06
ATOM	1797	O	HOH	327	36.760	29.941	31.666	1.00	22.03
ATOM	1864	O	HOH	328	15.305	36.513	15.694	1.00	39.62
ATOM	1798	O	HOH	329	33.005	46.924	36.994	1.00	22.07
ATOM	1863	O	HOH	330	23.801	36.154	22.715	1.00	46.30
ATOM	1799	O	HOH	331	33.609	31.296	26.261	1.00	23.85

FIG 5-28

```
ATOM   1862  O   HOH   332      34.942  24.780  29.532  1.00 38.93
ATOM   1800  O   HOH   333      25.235  12.919  54.611  1.00 36.20
ATOM   1861  O   HOH   334      38.048  23.467  36.645  1.00 37.73
ATOM   1801  O   HOH   335      12.284  43.511  38.338  1.00 33.79
ATOM   1802  O   HOH   336       9.826  47.020  32.568  1.00 46.67
ATOM   1803  O   HOH   337       7.671  41.532  29.806  1.00 40.88
ATOM   1804  O   HOH   338      15.430  23.713  26.808  1.00 34.73
ATOM   1805  O   HOH   339      24.344  20.385  25.121  1.00 53.42
ATOM   1806  O   HOH   340      31.550  10.656  40.819  1.00 47.85
ATOM   1807  O   HOH   341      17.569  23.030  25.796  1.00 28.17
ATOM   1808  O   HOH   342      19.174  38.552  23.965  1.00 45.54
ATOM   1809  O   HOH   343      24.268  37.527  25.415  1.00 30.97
ATOM   1810  O   HOH   344      21.266  29.482  41.551  1.00 19.69
ATOM   1811  O   HOH   345      20.668  26.999  41.933  1.00 11.81
ATOM   1812  O   HOH   346      24.780  24.795  43.460  1.00 20.95
ATOM   1813  O   HOH   347      42.962  13.170  46.312  1.00 31.00
ATOM   1814  O   HOH   348      32.322  14.088  47.013  1.00 28.20
ATOM   1815  O   HOH   349      31.708  13.186  49.679  1.00 35.57
ATOM   1816  O   HOH   350      22.408  35.801  50.514  1.00 40.71
ATOM   1817  O   HOH   351      25.366  47.090  42.583  1.00 38.15
ATOM   1818  O   HOH   352      27.243  47.647  43.977  1.00 41.55
ATOM   1819  O   HOH   353      29.868  45.076  42.906  1.00 29.32
ATOM   1820  O   HOH   354      14.175  22.269  42.680  1.00 74.11
ATOM   1821  O   HOH   355      13.414  10.739  35.791  1.00 29.92
ATOM   1822  O   HOH   356      20.338   9.974  37.765  1.00 30.46
ATOM   1823  O   HOH   357      23.520  40.420  24.953  1.00 29.75
ATOM   1824  O   HOH   358      25.718  41.692  26.023  1.00 30.43
ATOM   1825  O   HOH   359      26.826  38.466  25.345  1.00 31.72
ATOM   1826  O   HOH   360      37.768  42.373  25.123  1.00 41.53
ATOM   1827  O   HOH   361      40.078  42.268  25.852  1.00 37.12
ATOM   1828  O   HOH   362      31.483  38.677  22.083  1.00 54.21
ATOM   1829  O   HOH   363      33.891  37.723  30.126  1.00 23.35
ATOM   1860  O   HOH   364      39.936  26.543  36.329  1.00 47.93
ATOM   1830  O   HOH   365      36.631  34.210  41.636  1.00 62.74
ATOM   1831  O   HOH   366      37.038  29.783  52.197  1.00 40.07
ATOM   1832  O   HOH   367      37.289  37.407  40.231  1.00 37.59
ATOM   1833  O   HOH   368      18.930  17.517  52.472  1.00 35.80
ATOM   1834  O   HOH   369      19.506  18.914  57.913  1.00 45.72
ATOM   1835  O   HOH   370      30.903  26.708  41.139  1.00 21.54
ATOM   1836  O   HOH   371      30.369  25.678  24.583  1.00 22.46
ATOM   1837  O   HOH   372      21.000  33.705  20.826  1.00 26.00
ATOM   1838  O   HOH   373      13.648  32.794  21.329  1.00 27.98
ATOM   1839  O   HOH   374      29.735  25.683  38.707  1.00 21.00
ATOM   1859  O   HOH   375      33.670  24.419  60.503  1.00 50.04
ATOM   1840  O   HOH   376      30.034  11.047  37.420  1.00 43.28
ATOM   1841  O   HOH   377       8.662  35.846  35.068  1.00 51.94
ATOM   1842  O   HOH   378      10.847  36.466  39.503  1.00 42.32
ATOM   1843  O   HOH   379      14.395  48.943  39.085  1.00 29.72
ATOM   1844  O   HOH   380      36.676  11.660  40.172  1.00 39.81
ATOM   1845  O   HOH   381      35.968   7.212  34.763  1.00 58.66
ATOM   1846  O   HOH   382      17.426  21.988  21.077  1.00 41.69
ATOM   1847  O   HOH   383      29.837  22.623  39.378  1.00 32.82
ATOM   1848  O   HOH   384      23.855  29.386  55.164  1.00 55.00
ATOM   1849  O   HOH   385      17.408  35.360  47.495  1.00 61.61
ATOM   1850  O   HOH   386      27.900  49.720  42.448  1.00 47.70
ATOM   1851  O   HOH   387      13.932  36.230  44.385  1.00 45.08
ATOM   1852  O   HOH   388      12.650  28.021  43.288  1.00 49.86
ATOM   1853  O   HOH   389      16.974  42.367  43.435  1.00 34.38
ATOM   1854  O   HOH   390      37.335  42.653  28.295  1.00 64.46
ATOM   1855  O   HOH   391      29.701  49.856  35.323  1.00 62.61
ATOM   1856  O   HOH   392      27.267  50.835  33.976  1.00 66.60
ATOM   1857  O   HOH   393      19.661  29.181  51.537  1.00 34.01
ATOM   1858  O   HOH   394      29.412  17.505  59.089  1.00 51.78
TER
END
```


SPEX
3 02
10CND1N.SPT 10c non-mut, norm=1ex498
10CND2N.SPT 10c non-mut, norm=1ex498
Q69KMN.SPT Q69K-8, norm=1ex498
Q69KXN.SPT Q69K-8, norm=1em544
10C excitation
20A emission
10C emission
20A excitation
528.000 nm
0.9974
Normalized Intensity (cps)
1.0
0.8
0.6
0.4
0.2
0.0
450
500
528
550
600
Wavelength (nm)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14593

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/04; C07K 14/00, 16/00; C12N 1/20, 15/00, 15/09, 15/63

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/252.3, 252.33, 325, 410, 320.1; 530/350, 387; 536/23.1, 23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	WO 96/23810 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 08 August 1996, abstract and claims.	46–53, 88, 92, 93, 94 ---------- 1-41, 54-7
X ---- Y	HEIM et al. Improved green fluorescence. Nature. Vol. 373, 23 February 1995, pages 663-664, see Figure on page 664.	88 ---------- 2, 3, 10-16, 18-26, 28-32, 34-37, 39-41

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 18 DECEMBER 1997	Date of mailing of the international search report 27 JAN 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer NASHAAT T. NASHED Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14593

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	HEIM et al. Wavelength mutations and posttranslational autoxidation of green fluorecent protein, Proc. Natl. Acad. Sci. USA. Vol. 91, December 1994, pages 12501-12504, see abstract.	46, 48, 50, 52, 54, 56, 94 ---------- 1-41, 47, 49, 51, 53, 55, 57
Y	PEROZZO et al. X-ray diffraction and time-resolved fluorescence analysis of Aequorea green fluorescent crystals. Journal of Biological Chemistry. 05 June 1988, Vol. 263, No. 16, pages 7713-7716.	1-41, 46-57, 86-94
X ----- Y	DELAGRAVE et al. Red-shifted excitation mutants of the green fluorescent protein. Bio/Technology. February 1995, Vol. 13, pages 151-153, see Table 1 on page 152.	46-57, 86-88, 91 ---------- 1-41
Y	EHRIG et al. Green-fluorescent protein mutants with altered fluorescence excitation spectra. FEBS Letters. 1995, Vol. 367, pages 163-166, abstract.	1-41, 46-57, 89, 90
Y	WANG et al. Implication for bcd mRNA localization from spatial distribution of exu protein in Drosophila oogenesis. Nature. 02 June 1994, Vol. 369, 400-403, see Figure 1.	32-41, 54-57
P, Y	ORMO et al. Crystal structure of the Aquorea victoria green fluorescent protein. Science. 06 September 1996, Vol. 273, pages 1392-1395, abstract.	1-41, 46-57, 86-94
P, Y	YANG et al. The molecular structure of green fluorescent protein. Nature Biotechnology. October 1996, Vol. 14, pages 1246-1251, abstract.	1-41, 46-57, 86-94
P, Y	PALM et al. The structural basis for spectral variations in green fluorescent protein. Nature Struct. Biol. May 1997, Vol. 4, Number 5, pages 361-365.	1-41, 46-57, 86-94
A	US 5,491,084 A (CHALFIE et al.) 13 February 1996, entire document.	1-41, 46-57, 86-94

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14593

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-41, 46-57, 86-94

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14593

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/252.3, 252.33, 325, 410, 320.1; 530/350, 387; 536/23.1, 23.4

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN: Medline, Caplus, Scisearch, Lifesci, Biosis, Embase, Wpids, Biotechds.
Search terms: aequorea and green fluorescent, T-203, Thr-203, T203, DNA, cDNA, sequence, s65t, t203h, s65t, t203y, s72a, f64l, s65g, 203y, s72a, s65g, v68l, t203y, l42x, v61x, t62x, v68x, q69x,n121x,y145x, v150x, f165x, i167x, q183x

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-41, drawn to DNA coding for mutant fluorescent green protein having mutation at Thr-203, the fluorescent protein, antibody labeled with the fluorescent protein and the DNA coding for a fusion protein consisting of an antibody and the mutant fluorescent protein.

Group II, claims 42-45 and 58-61, drawn to a DNA probe labled with mutants fluorescent.

Group III, claims 46-57 and 86-94, drawn to drawn to DNA coding for mutants fluorescent green protein having mutation at an amino acid residue other than Thr-203, the fluorescent protein, antibody labeled with the fluorescent protein and the DNA coding for a fusion protein consisting of an antibody and the mutant fluorescent protein.

Group IV, claims 62-64 and 68-70, drawn to a method for engineering fluorescent protein.

Group V, claims 65-67, drawn to method of producing fluorescent resonance energy transfer.

Group VI, claims 71-74, drawn to a fluorescent protein crystal having the amino acid sequence SEQ ID NO: 2.

Group VII, claims 75-82, drawn to a computation method for the design of fluorescent protein.

Group VIII, claims 83-85, drawn to a storage device containing the atomic coordinate.

Group IX, claims 95-100, drawn to a method of identifying test chemicals.

The inventions listed as Groups I-IX do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Each of the above group has a special technical feature defined by the first claim in the Group. The following are the special technical feature for each Groups: (a) Group I is the nucleic acid coding for fluorescent protein having at least the mutation at Thr-203, (b) Group II is a fluorescent DNA probe labeled with a mutant fluorecent protein, (c)Group III is the nucleic acid sequence coding for mutant fluorescent protein having mutation at residues other than T-203, (d) Group IV is a method for the engineering of mutant fluorescent proteins, (e) Group V is a method for producing fluorescent resonance energy transfer, (f) Group VI is the protein crystal of the wild-type protein, (g) Group VII is the computation method to design mutants fluorescent protein with different fluorescent characteristics, (h) Group VIII is a storage device for data, and (i) Group IX is a method of identifying test chemicals.

Group I encompasses the nucleic acid coding for the mutant fluorescent protein, expression vector, recombinant host cell, the mutant proteins and a use for the DNA in making the fusion protein consisting of antibody and the fluorescent protein. Group II represent a second use for the mutant protein of Group I. Also, the special technical feature of Group I is different from that of Group III because the DNA of each Group codes for different sets of mutants that do not share common feature. The special technical feature for this Group I is distinct from those of Groups IV-IX.

The special technical feature of Group II, the fluorescent DNA probe is clearly different from those of Groups III-IX. The DNA probe of Group II represent a second use of the fluorescent protein of Group II.

The method of engineering fluorescent protein of Group IV is different from that of producing fluorescent resonance energy transfer of Group V because the resulting fluorescence is different in each case and vary in its characteristics. Similarly, the special technical features of each of Groups IV and V are different from those of the crystal of Group VI, the computation method of Group VII, the storage device of Group VIII, and the method of identifying chemicals of Group IX. Finally, the crystal of Group VI, the computation method of Group VII, the storage device of Group VIII, and the method of identifying chemicals of Group IX are clearly unrelated to each other and there is no special technical feature that connects them together.